MEASURE DHS

Biomarker Field Manual

[COUNTRY] Demographic and Health Survey

[IMPLEMENTING AGENCY]

ICF International / Demographic and Health Surveys
Calverton, Maryland
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MEASURE DHS is a five-year project to assist institutions in collecting and analyzing data needed to plan, monitor, and evaluate population, health, and nutrition programs. MEASURE DHS is funded by the U.S. Agency for International Development (USAID). The project is implemented by ICF Macro in Calverton, Maryland, in partnership with the Johns Hopkins Bloomberg School of Public Health/Center for Communication Programs, the Program for Appropriate Technology in Health (PATH), Futures Institute, Camris International, and Blue Raster.

The main objectives of the MEASURE DHS program are to: 1) provide improved information through appropriate data collection, analysis, and evaluation; 2) improve coordination and partnerships in data collection at the international and country levels; 3) increase host-country institutionalization of data collection capacity; 4) improve data collection and analysis tools and methodologies; and 5) improve the dissemination and utilization of data.

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PREFACE

In combination with classroom instruction and practical experience, this manual will be used to teach you how to collect biomarkers for the [YEAR] [COUNTRY] Demographic and Health Survey (DHS). Before each training session, you should carefully study this manual and the biomarker section of the household questionnaire. You are encouraged to ask questions during training and to discuss problems encountered in order to avoid making mistakes during fieldwork. The stages of training are described below:

- During the first phase, we will review with you the chapters of this manual. You will learn how to identify eligible respondents, record information relating to biomarker collection in the household questionnaire or on special field forms, handle the technical procedures involved in height/length and weight measurement and, in blood collection, testing, and transportation, and other related instructions.

- In the second phase, you will practice the procedures you've been taught by role playing with other trainees. This practice will include finger pricks for anemia testing [and DBS collection for HIV testing].

- In the third phase, you will visit a clinic or a health center and, with their parent's consent, practice collecting biomarkers from infants and young children.

- In the final phase, known as field practice, you will be assigned to a [COUNTRY] DHS trainee team. During field practice, you will collect biomarkers from eligible children and adults exactly as you will during the survey. Households that you visit will be in clusters that are not part of the [COUNTRY] DHS sample.

Throughout the training, you may be given homework assignments and tests. At the end of the training, your overall performance will be assessed and those who have performed the best will be selected to work in the survey.

Your training does not end at the start of fieldwork. Rather, it is a continuous process. Your team supervisor and the [COUNTRY] DHS health and survey coordinators will play important roles in continuing your training and in ensuring the quality of data you collect throughout the survey. They will:

- Periodically observe your fieldwork activities to ensure that you are conducting yourself professionally, obtaining informed consent from respondents, and following the biomarker collection protocol correctly;

- Spot check that you collected biomarkers from the correct households and only from eligible respondents;

- Collect blood specimens for transport to the laboratory and consolidate the field record forms;

- Regularly meet with you to discuss your performance and give out future work assignments.

Any field staff member who is not performing at the level necessary to produce the high quality data required to make the [COUNTRY] DHS a success may be released from service.

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CHAPTER 1: GENERAL INFORMATION

OVERVIEW OF BIOMARKER COLLECTION

A biomarker may be thought of as a characteristic that can be independently measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response to a therapeutic intervention[1]. Biomarker measurements can serve as diagnostic tools to identify diseases in their early stages and can be used as surveillance tools to track changes in disease patterns or to evaluate intervention programs. In population-based surveys, biomarkers help assess the prevalence or occurrence of diseases or conditions and can also be used at a macro level to measure the long-term effect of policies and programs. In the DHS, biomarkers are collected in order to report levels of specific disease and conditions on a population level. Specific to the [YEAR] [COUNTRY] DHS, the following biomarkers will be collected/tested: [anthropometry], [anemia], [HIV], and [OTHER BIOMARKERS]. This training manual will discuss the proper collection techniques and the appropriate recording and result reporting of these biomarkers.

Biomarker collection should take place after the completion of the household and individual questionnaires. However, prior to collection, certain tasks must be completed. This chapter reviews the tasks that need to be completed prior to collecting biomarkers:

- Determining eligibility
- Obtaining informed consent.

[1] Biomarker Definitions Working Group, National Institutes of Health, 2001

ELIGIBILITY

Not all household members are eligible for biomarker collection. Members of the household who are eligible for biomarker collection are: women age 15-49, men age 15-[49], and children under age 6 years old who are usual household residents or are visitors that have stayed in the house the night before the household interview took place. **It is the responsibility of the interviewer** to identify all of the household members eligible for biomarker collection and to enter their names and line numbers into the [biomarker pages at the end of the Household Questionnaire (if using paper questionnaires) or Biomarker Data Form (if using electronic questionnaires)]. Individuals eligible for biomarker collection will be identified by reviewing these columns from the Household Schedule:

- Column (1) Line number
- Column (2) Name
- Column (4) Sex of household member
- Column (7) Age of household member
- Column (9) Identification of eligible women, (women age 15-49)
- Column (10) Identification of eligible men, (men age 15-[49])
- Column (11) Identification of eligible children, (children under age 6)

HOUSEHOLD SCHEDULE

							IF AGE 15 OR OLDER			
LINE NO.	USUAL RESIDENTS AND VISITORS	RELATIONSHIP TO HEAD OF HOUSEHOLD	SEX	RESIDENCE		AGE	MARITAL STATUS	ELIGIBILITY		
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	Please give me the names of the persons who usually live in your household and guests of the household who stayed here last night, starting with the head of the household. AFTER LISTING THE NAMES AND RECORDING THE RELATIONSHIP AND SEX FOR EACH PERSON, ASK QUESTIONS 2A-2C TO BE SURE THAT THE LISTING IS COMPLETE. THEN ASK APPROPRIATE QUESTIONS IN COLUMNS 5-20 FOR EACH PERSON.	What is the relationship of (NAME) to the head of the household? SEE CODES BELOW.	Is (NAME) male or female?	Does (NAME) usually live here?	Did (NAME) stay here last night?	How old is (NAME)? IF 95 OR MORE, RECORD '95'.	What is (NAME)'s current marital status? 1=MARRIED OR LIVING TOGETHER 2=DIVORCED/ SEPARATED 3=WIDOWED 4=NEVER-MARRIED AND NEVER LIVED TOGETHER	CIRCLE LINE NUMBER OF ALL WOMEN AGE 15-49	CIRCLE LINE NUMBER OF ALL MEN AGE 15-49	CIRCLE LINE NUMBER OF ALL CHILDREN AGE 0-5
01			M F 1 2	Y N 1 2	Y N 1 2	IN YEARS		01	01	01
02			1 2	1 2	1 2			02	02	02

After referencing the Household Schedule, the interviewer will record the names and line numbers of people eligible for biomarker collection in the [biomarker pages at the end of the Household Questionnaire (if using paper questionnaires) or Biomarker Data Form (if using electronic questionnaires)]. The interviewer will also record the dates of birth of children in Question 203. For a child whose mother was interviewed, the month and year of the child's birth should be taken from the mother's birth history (recorded in the Woman's Questionnaire), while the day of birth must be obtained directly from the mother. For a child whose mother has not been interviewed or is unavailable, the full date of birth must be obtained from a responsible adult.

WEIGHT, HEIGHT AND HEMOGLOBIN MEASUREMENT FOR CHILDREN AGE 0-5

201	CHECK COLUMN 11 IN HOUSEHOLD SCHEDULE. RECORD THE LINE NUMBER AND NAME FOR ALL ELIGIBLE CHILDREN 0-5 YEARS IN QUESTION 202. IF MORE THAN SIX CHILDREN, USE ADDITIONAL QUESTIONNAIRE(S).			
		CHILD 1	CHILD 2	CHILD 3
202	LINE NUMBER FROM COLUMN 11 NAME FROM COLUMN 2	LINE NUMBER . . . . . ☐☐ NAME _____	LINE NUMBER . . . . . ☐☐ NAME _____	LINE NUMBER . . . . . ☐☐ NAME _____
203	IF MOTHER INTERVIEWED, COPY MONTH AND YEAR OF BIRTH FROM BIRTH HISTORY AND ASK DAY; IF MOTHER NOT INTERVIEWED, ASK: What is (NAME)'s birth date?	DAY . . . . . ☐☐ MONTH . . . . . ☐☐ YEAR ☐☐☐☐	DAY . . . . . ☐☐ MONTH . . . . . ☐☐ YEAR ☐☐☐☐	DAY . . . . . ☐☐ MONTH . . . . . ☐☐ YEAR ☐☐☐☐
204	CHECK 203: CHILD BORN IN JANUARY 2005 **(9)** OR LATER?	YES . . . . . 1 NO . . . . . 2 (GO TO 203 FOR NEXT CHILD OR, IF NO MORE CHILDREN, GO TO 214)	YES . . . . . 1 NO . . . . . 2 (GO TO 203 FOR NEXT CHILD OR, IF NO MORE CHILDREN, GO TO 214)	YES . . . . . 1 NO . . . . . 2 (GO TO 203 FOR NEXT CHILD OR, IF NO MORE CHILDREN, GO TO 214)

The [Household Questionnaire/Biomarker Data Form] is then given to the health specialist to record all data related to biomarker collection. It is the responsibility of the health specialist to verify that the information recorded by the interviewer is properly recorded prior to collecting any biomarkers. For children under 6 years, verify the child's name and line number is written in Question 202. Verify that an exact date of birth (day/month/year) is written in Question 203. Proceed with Question 204.

For women, verify the line number and name of all women age 15 - 49 in Question 215, and for men, verify the line number and name all men age 15 - [49] in Question 244.

WEIGHT, HEIGHT, HEMOGLOBIN MEASUREMENT AND HIV TESTING FOR WOMEN AGE 15-49

214	CHECK COLUMN 9 IN HOUSEHOLD SCHEDULE. RECORD THE LINE NUMBER AND NAME FOR ALL ELIGIBLE WOMEN IN 215. IF THERE ARE MORE THAN THREE WOMEN, USE ADDITIONAL QUESTIONNAIRE(S).			
		WOMAN 1	WOMAN 2	WOMAN 3
215	LINE NUMBER FROM COLUMN 9 NAME FROM COLUMN 2	LINE NUMBER . . . . . ☐☐ NAME _____	LINE NUMBER . . . . . ☐☐ NAME _____	LINE NUMBER . . . . . ☐☐ NAME _____

The table below summarizes which household members are eligible for measurements and tests.

Groups eligible for biomarker collection*	**[Weight]**	**[Height/ length]**	**[Anemia testing]**	**[DBS collection for HIV testing]**
Children age 0-5 months	X	X		
Children age 6-71 months	X	X	X	
Women age 15-49 years	X	X	X	X
Men age 15-[49] years	X	X	X	X

*Must be usual residents or have slept in the household the night before the household interview took place.

The following are important points to keep in mind when completing the Biomarker Data Form:

1) Biomarker data should be collected from eligible consenting adults *after* their individual interview has been completed.

2) Collect biomarkers from one individual at a time. All biomarker information collected for the [COUNTRY] DHS should be collected on one individual before moving on to the collection of biomarkers from the next eligible, consenting person. For example, if there is more than one eligible woman in a household who has consented to biomarker collection, weigh and measure her, test her for anemia, and collect blood for HIV testing *before* proceeding to the next woman. Likewise, complete the collection of all biomarkers from one child before proceeding to the next. Failure to do so may lead to results being recorded in the wrong columns of the questionnaire.

3) Never alter any responses on the Household Questionnaire without consulting the interviewer (if you were not the interviewer for the Household Questionnaire). Even in cases where there are concerns about an individual's eligibility for testing, proceed with [anemia testing] and/or [HIV test blood sample collection]. Record in the comments section of the Household Questionnaire a description of the problem. Provide as many details as possible. The field organization/central office will decide later what will be done about the test results for the respondent in question.

INFORMED CONSENT

One of the primary tasks before biomarker collection is to explain the purpose of the testing to eligible respondents or, in the case of children, to the parent/responsible adult, and to obtain their consent before collecting any blood samples. In order to ensure that these individuals can make an "informed" decision about whether or not to be tested, the [COUNTRY] DHS questionnaire includes consent statements which you will read to the respondent. These consent statements include the following basic elements:

- a description of the objectives of the test
- basic information on how the test will be conducted
- assurances about the confidentiality of the results
- a specific request for permission to collect the sample

You must read the informed consent statements to each eligible respondent age 18 and over and obtain the respondent's consent to the testing before any blood collection is done. The approach for obtaining consent differs slightly when the eligible individual is a child under age 6 years or an adolescent age 15-17 years.

If the eligible individual is a child, you must obtain the consent of one of the child's parents, or, in the absence of a parent, the consent of a responsible adult who is at least 18 years of age. If the parent/responsible adult does not consent to the test, the test must not be performed.

If the eligible individual is an adolescent (age 15-17 years), you must first obtain the consent of the adolescent's parent or responsible adult. If the parent of responsible adult gives their consent, you will then seek the consent of the adolescent. If either the parent/responsible adult or the adolescent does not consent to the test, the test must not be performed. There are two exceptions to this rule: 1) if the adolescent is married (or was formerly married) or 2) if the adolescent lives alone or in a household in which there are no adults. In either instance, the adolescent is considered an emancipated minor, and is to be treated like an adult. In this case, consent of the adolescent is sufficient.

Prior to performing the anemia test or collecting blood for HIV testing, you must record the outcome of the consent request on the Biomarker Data Form. This is discussed in more detail in the upcoming chapters. You must also sign your name to indicate that you read the consent statement to the respondent, or in the case of children, to the parent/responsible adult and have recorded their response accurately.

Key points to remember include:

1. **Read the applicable consent statements to each eligible respondent exactly as they appear in the questionnaire**. When you arrive at the household and begin talking about the blood tests with the respondent, you may informally discuss many of the items included in the informed consent statement. However, before beginning the testing procedures, you must still read the informed consent statements exactly as they are worded in the questionnaire. If you feel that the respondent may find the statements repetitive, tell him/her that you are required to

read the statement to ensure that respondents are given all the appropriate information.

2. **Read the informed consent statements clearly.** Practice reading the consent statements out loud so that you become comfortable delivering them in a clear, natural voice and manner. Avoid speaking rapidly or in a monotone.

3. **For adults and adolescents, always request consent for the anemia and HIV testing separately.** Be sure the respondent knows that it is possible to consent to one test and not to the other. Since the outcome of the consent process may differ for the two tests, it is important that you accurately record the results for both the anemia and HIV testing.

4. **Never collect blood from an adolescent before obtaining the consent of the parent/responsible adult** unless the adolescent is married, was formerly married, lives alone or lives in a household where there are no adult members.

5. **Never attempt to force or coerce consent.** Some respondents may be suspicious or fearful of having their blood collected for testing. Others may have questions or want to discuss the procedures before giving consent. Take time to patiently respond to all questions.

6. Some respondents may be reluctant to allow testing without consulting someone not present at the time of your visit (for example, a woman may want to consult her husband before giving permission). **In such cases, make an appointment to return to the household later at an agreed upon time.** If you believe it will help, ask the team supervisor to visit a household where eligible respondents express fear or reluctance to be tested.

Once the individual interviews have been completed, eligible respondents have been identified, and consent has been obtained, biomarker collection can take place.

Summary of Steps in Identifying Eligible Respondents and Seeking Informed Consent for Blood Collection

- Complete the household and individual questionnaires
- Look at the Household Listing Schedule to confirm individuals who are eligible for biomarker collection:
    - Adults, age 15-49, who are usual residents or who stayed in the household the night before the interview are eligible for biomarker collection
    - Children, under age 6, who are usual residents or who stayed in the household the night before the interview, are eligible for biomarker collection
        - Children 6 months - under 6 years: eligible for anthropometry and blood collection
        - Children below 6 months: eligible for anthropometry only

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- For adults, age 18- 49: Obtain consent for blood collection as follows
    - Read consent exactly as written;
    - Record the outcome of the consent request and sign in the space provided;
    - If consent is granted, proceed with blood collection.

- For never-married adolescents, age 15-17:
    - Obtain consent for blood collection from parent or responsible adult
        - Read consent exactly as written
        - Record whether the parent/responsible adult consented or refused and sign in the space provided;
    - If the parent/responsible adult consented, obtain consent for blood collection from adolescent
        - Read consent exactly as written;
        - Record the outcome of the consent request and sign in the space provided;
        - If consent was granted, proceed with blood collection.

- For children 6 months-6 years:
    - Obtain consent for blood collection from parent or responsible adult
        - Read consent exactly as written;

CHAPTER 2: ANTHROPOMETRY

Anthropometry refers to the measurement of humans. In the [COUNTRY] DHS, anthropometry refers solely to the measurement of a person's height (length) and weight. This information can be used to assess the nutritional status of a population. For children, standard indices of physical growth related to nutritional status are height-for-age, weight-for-height, and weight-for-age. A child who is below minus two standard deviations (-2 SD) from the median of a reference population in terms of height-for-age is considered short for his/her age or stunted. Stunting reflects the cumulative effect of chronic malnutrition. A child who is below minus two standard deviations (-2 SD) from the median of a reference population in terms of weight-for-height is considered too thin for his/her height, or wasted. Wasting is a condition reflecting acute or recent nutritional deficit. Weight-for-age is a composite index of stunting and wasting and is a good indicator to monitor nutritional status over time.

Among adults, height and weight measurement are used to calculate a person's body mass index (BMI) and to assess a woman's risk of having difficulty during childbirth due to her short stature (height <145 cm). BMI is calculated by dividing the weight in kilograms by the height in meters squared (kg/m^2). BMI values are used to determine the percentage of the adult population that is normal, thin, overweight and obese.

MATERIALS AND EQUIPMENT FOR ANTHROPOMETRY

- **SECA 874 digital scale[2]:** for weighing children and adults. The scale has a 200 kg capacity and weighs in 0.01 kg increments. The scale is powered by six AA batteries and has an "ON-OFF" switch located at the side of the scale.

- **Shorr height board:** for measuring the height (length) of children and adults.

- **Household Questionnaire with Biomarker Data Form**

PROCEDURES AND PRECAUTIONS BEFORE MEASURING

1. **Layout of the Procedures**: Each step of the measurement procedure is directed at specific participants, who are named in bold letters at the beginning of each step: "**Measurer**" and "**Assistant**".

2. **Two Trained People Required**: Two trained people are required to measure a child's height or length. The measurer holds the child and takes the measurements. The assistant helps hold the child and records the measurements on the questionnaire. If there is an untrained assistant such as the mother, then the trained measurer should also record the measurements on the questionnaire. One person alone can take the weight of a child and record the results if an assistant is not available.

[2] The Seca 874 digital floor scale is manufactured by Seca gmbh & co. kg. Hammer Steindamm 9 – 25. 22089 Hamburg. Germany. The scale can be procured directly from Seca. These instructions were adapted from instructions that accompany the scale and revised by Irwin J. Shorr, MPH, MPS.

3. **Measuring Board and Scale Placement:** Be selective about where you place the measuring board and scale. It is best to measure outdoors during daylight hours. If it is cold, raining or if too many people congregate and interfere with the measurements, it may be more comfortable to weigh and measure indoors. Make sure there is adequate light.

4. **Age Assessment:** Before you measure, determine the child's age. If the child is less than two years, measure length (the child lying down). If the child is two years of age or older, measure height (the child is standing). If accurate age is not possible to obtain, measure length if the child is less than 85 cm. Measure height if the child is equal to or greater than 85 cm.

5. **Weigh and Measure One Child at a Time:** If there is more than one eligible child in a household, complete the weighing and measuring of one child at a time. Then proceed with the next eligible child. DO NOT weigh and measure all the children together. If there is more than one eligible woman in a household, weigh and measure her and all her eligible children before proceeding with the next woman. Otherwise measurements may get recorded in the wrong columns of the questionnaire. Return measuring equipment to the storage bags after you complete the measurements for each household.

6. **Control the Child:** When you weigh and measure, you must control the child. The strength and mobility of even very young children should not be underestimated. Be firm yet gentle with children. Your own sense of calm and self confidence will be felt by the parent and the child.

   When a child has contact with a measuring board, you must hold and control the child so the child will not trip or fall. Never leave a child alone with a piece of equipment.

7. **Coping with stress:** Since weighing and measuring requires touching and handling children, normal stress levels for this type of survey work are higher than for surveys where only verbal information is collected.

   Explain the weighing and measuring procedures to the mother, father, or other responsible adult and to a limited extent, the child, to help minimize possible resistance, fears or discomfort they may feel. You must determine if the child or the parent is under so much stress that the weighing and measuring must stop. Remember, young children are often uncooperative; they tend to cry, scream, kick and sometimes bite. If a child is under severe stress and is crying excessively, try to calm the child or return the child to the parent before proceeding with the measuring.

   Do not weigh or measure a child if:

   - The parent/responsible adult refuses.
   - The child is too sick or distressed.
   - The child is physically deformed which will interfere with or give an incorrect measurement. To be kind, you may want to measure such a child and make a note of the deformity on the questionnaire.

8. **Recording Measurements and Being Careful:** Keep objects out of your hands and pens out of your mouth, hair or breast pocket when you weigh and measure so that neither the child nor you will get hurt due to carelessness. When you are not using a pen, place it in your equipment pack or on the questionnaire. Make sure you do not have long fingernails. Remove interfering rings and watches before you weigh and measure.

9. **Strive for Improvement:** You can be an expert measurer if you strive for improvement and follow every step of every procedure the same way every time. The quality and speed of your measurements will improve with practice. You will be required to measure women, men, and children. Do not take these procedures for granted even though they may seem simple and repetitious. It is easy to make errors when you are not careful. Do not omit any steps. Concentrate on what you are doing.

STEPS FOR MEASURING WEIGHT AND HEIGHT/LENGTH

1. Check column 11 of the household schedule to identify all children in the household who are eligible for weight and height/length measurement. Children born in [YEAR] or later who are usual residents of the household or who are visitors who spent the previous night in the household are eligible for anthropometry. Each eligible child should be recorded in a separate column of the **Biomarker Data Form**.

2. Referring to the household schedule, check the **Line Number** and **Name** of all eligible children in **Question 202**. In **Question 203,** verify the month and year of birth of the child from the mother's birth history (Woman's Questionnaire Section 2), and ask for the day of birth. For children not included in a birth history, determine the date of birth from the parent/responsible adult and record in **Question 203.**

3. In **Question 204** confirm that the child was born in [MONTH YEAR] or later. If the child was born before [MONTH YEAR], go to **Question 203** for the next child.

4. Perform the child weight and height/length measurements according to instructions below.

5. Next, by checking columns 9 and 10 of the household schedule, identify all women and men from the household schedule who are eligible for weight and height measurement. [Women age 15-49 years] and [men age 15-49 years] who are usual members of the household or who slept in the household the night before are eligible.

6. Check that the **Line Number** and **Name** of all eligible women and men has been recorded in **Question 215** and **Question 244**, respectively, of the **Biomarker Data Form**.

7. Perform the adult weight and height measurements according to instructions below.

MEASURING WEIGHT

Preparing Adult and Child to Take Their Weight

Show the scale to the adult and explain that you will weigh her/him and their children on the scale. Tell her/him that infants and any other children who will not stand on the scale alone can be held by the adult to obtain the child's weight. Ask the adult to wear light clothing while being weighed and to remove shoes/sandals and any heavy clothing, etc. Ask the adult to undress the child just before taking his/her weight. Leave underpants on the child.

Preparing the Scale

Take the scale out of the storage bag and place the scale on a hard, level surface. Uneven surfaces or vibration may cause the scale to malfunction. Turn on the power by pushing the switch located at the side to the "ON" position.

The scale will not function correctly if it is bumped, knocked or moved during the weighing. It is best to use the scale in the shade or indoors. Handle the scale carefully:

- Do not drop or bump the scale.
- Do not weigh a total weight of more than 200 kg.
- Do not store the scale in direct sunlight or other hot places.
- Protect the scale against excess humidity or moisture.
- Do not use the scale at temperatures below +10°C or above +40°C.
- To clean the scale, wipe surfaces with a damp cloth and dry immediately
- Never put the scale in water.
- After using the scale, turn off the scale by switching the "ON-OFF" switch to the "OFF" position.

<u>Weighing Adults and Children Who Can Stand on the Scale by Themselves</u>

1. Activate the power supply by pushing the switch in position "ON". Press the "START" key with no load on the scale. The display should show "SECA, 8.8.8.8.8 and "0.00" consecutively. The scale automatically sets to zero "0.00" and is now ready for use.

2. Ask the adult or child to step onto the center of the scale and stand quietly. Wait until the numbers on the display no longer change and stay fixed in the display.

3. The weight will appear in the digital display. Record the weight to 0.01 kg on the questionnaire.

    - For children, record the child's **weight** measurement in **Questions 205.** If the child's weight was not measured, record the appropriate code in **Question 205.**

    - Record an adult's **weight** measurements in **Question 216/245**. If the adult's weight was not measured, record the appropriate code in **Question 216/245**

4. If you have just weighed an adult and you are about to weigh an infant or child that must be held to take its weight, then ask the adult to remain on the scale since the adult will hold the child.

Weighing Infants or Children Who Must be Held by an Adult While on the Scale
*(NOTE: If it is cold and the adult wants the child to be covered during the weighing, give her/him a blanket or cloth for covering the child **after** you have recorded the adult's weight on the **Biomarker Data Form**. If you do not give the adult a blanket or cloth to cover the child, follow the instructions given under "**If You Do NOT Give the Adult a Blanket or Cloth to Cover the Child"**. If you give the adult a blanket or cloth to cover the child, follow the instructions given under "**If You Give the Adult a Blanket or Cloth to Cover the Child"**).*

If You Do NOT Give the Adult a Blanket or Cloth to Cover the Child:

2 in 1 Function

The 2 in 1 function enables the weight of babies and small children to be determined. The child is held in the arms of an adult. Proceed as follows.

1. Press the "START" key with no load on the scale and wait until the display "0.00" appears.

2. Ask the adult to step onto the center of the scale without the child and stand quietly. Wait until the numbers (weight of the adult) on the display no longer change and stay fixed in the display.

3. Press the 2 in 1 key to activate the function. The scale stores the weight of the adult and the display returns to zero. "0.00" and "NET" appear in the display.

4. Give the child to the adult. The scale determines the weight of the child. Once the value is stable for about 3 seconds, the display is retained. This avoids the display jumping about as a result of the child's movements. "HOLD" and "NET" appear in the display.

5. Record the weight of the child to 0.01 kg on the questionnaire.

6. If several children are to be weighed consecutively, it is important that it is always the same adult who performs the measurement and that this person's weight does not change (e.g. due to pieces of clothing being removed).

7. The 2 in 1 function remains switched on until:
   - You press the 2 in 1 key again (total weight displayed)
   - The scale switches off automatically.

If You Give the Adult a Blanket or Cloth to Cover the Child:

2 in 1 Function

1. Press the "START" key with no load on the scale. Wait until the display "0.00" appears.

2. **Give the adult a blanket or cloth and ask her/him to step onto the center of the scale without the child and stand quietly.**

3. The weight of the adult plus blanket/cloth appears on the display. Wait until the numbers on the display no longer change and stay fixed.

4. Press the 2 in 1 key to activate the function. The scale stores the weight of the adult plus blanket/cloth and the display returns to zero. “0.00” and “NET” appear in the display.

5. Give the child to the adult. The scale determines the weight of the child. Once the value is stable for about 3 seconds, the display is retained. This avoids the display jumping about as a result of the child’s movements. “HOLD” and “NET” appear in the display.

6. Record the weight of the child to 0.01 kg on the questionnaire.

7. If several children are to be weighed consecutively, it is important that it is always the same adult who performs the measurement and that this person’s weight does not change (e.g. due to pieces of clothing being removed).

8. The 2 in 1 function remains switched on until:
   - You press the 2 in 1 key again (total weight displayed)
   - The scale switches off automatically.

Additional Notes on the SECA scale**:**

- The SECA scale switches off automatically. If the scale is in “Normal Mode” it will switch off 3 minutes after the last weighing. If the scale is in “2 in 1 mode”, it will switch off after two minutes.

- Do not weigh loads with a total weight of more than 200 kg.

- Possible reasons for the scale not taring (returning to zero (“0.00”) after pressing the “2 in 1 function” key when the adult stands on the scale):

    - There was no weight on the scale to tare (the adult was not on the scale).

    - The load weighs more than 200 kg; “STOP” appears in the display. Use a lighter load.

What to do if the Scale Display Shows the Following Errors:

No weight is displayed when there is a load on the scale?

- Check to see if the scale is switched on. Ask the adult to step off the scale and step gently on the weighing platform.

- Check to see if the switch is in the “ON” position

- Check the batteries

The scale keeps switching on, while being transported? The vibration switch has been activated. Turn off the scale by switching the “ON-OFF” switch to the “OFF” position.

The scale displays a weight after being transported or after new batteries have been put in? Wait until the scale switches off automatically after two minutes. The scale will work normally again.

“*0.00*” does not appear before weighing? Start the scale again after it switches off automatically; there should not be any load on the scale.

“----” appears instead of “*0.00*” before weighing? Start the scale again after it switches off automatically; there should not be any load on the scale.

One segment of the display is illuminated constantly or not at all? There is a problem with that segment of the scale. Inform your service dealer.

The display shows a battery with split shading? The battery voltage is running low. The batteries should be changed in a few days.

“..*batt*” appears in the display? The batteries are empty. Replace the batteries.

“*Stop*” appears in the display? The maximum load capacity of the scale has been exceeded.

The display flashes? Take the load off the scale and start again. Wait until 0.00 appears and weigh again.

‘temp’ appears in the display? The ambient temperature of the scale is too high or too low. Place the scale in an ambient temperature between + 10ºC and + 40ºC. Wait 15 minutes for the scale to adapt to the temperature and weigh again.

E and a number appear in the display? Start the scale again after it switches off automatically. The scale will then work normally again. If this does not happen, inform the service dealer.

MEASURING A CHILD’S HEIGHT (ILLUSTRATION 1):

1. **Measurer or Assistant**: Place the Shorr measuring board on a hard, flat surface against a wall, table, tree or staircase. Make sure the measuring board is stable. Many walls and floors are not at perfect right angles; if necessary, place small rocks underneath the height board to stabilize it during the measurement.

2. **Measurer or Assistant**: Ask the parent to take off the child’s shoes and to unbraid or push aside any hair that would interfere with the height measurement. Ask the parent to bring the child to the measuring board

and to kneel in front of the child so that the child will look forward at the parent.

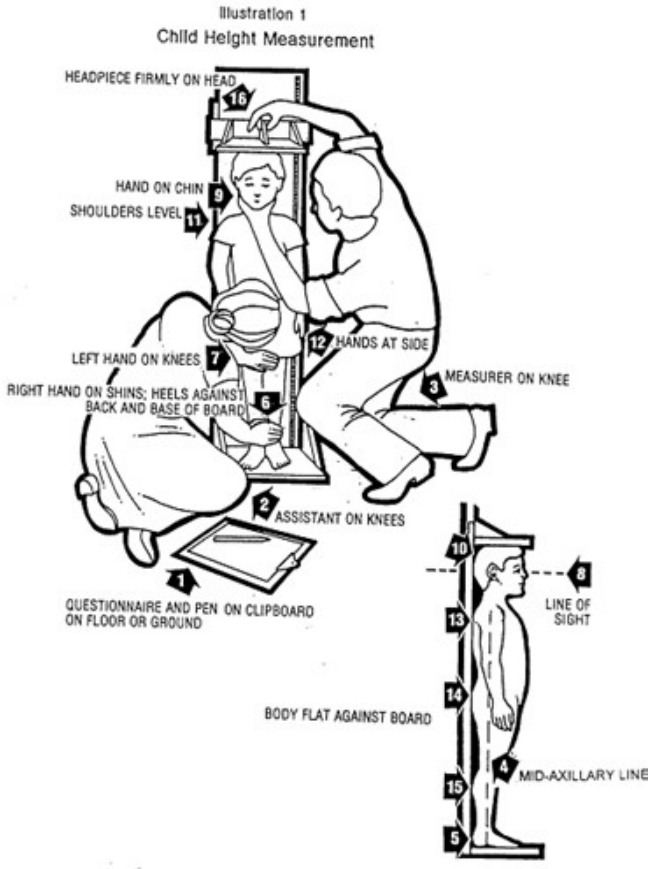
3. **Assistant**: Place the questionnaire and pen on the ground (Arrow 1) and kneel on the right side of the child (Arrow 2).

4. **Measurer**: Kneel on the left of the child (Arrow 3).

5. **Assistant**: Place the child's knees and feet in the correct position, with knees and feet either together or apart. There are three possible positions for the knees and feet:
   - Knees together and feet together
   - Knees together and feet apart
   - Knees apart and feet together

   } Whichever touches first!

6. **Measurer**: Determine if the child's feet should be against or away from the back of the height board by observing the imaginary line drawn from the tip of the shoulder to the heel, which is called the "mid-axillary line" (Arrow 4). This line should be perpendicular (90°) to the base of the height board where the child is standing (you may have to move the child's feet away from the back of the height board to put them in the proper position). Note that with most preschool-age children who are not heavy or obese, the heels will probably touch the back of the height board (Arrow 5).



7. **Assistant**: With your thumbs against the index finger of each hand, place your right hand on the child's shins (Arrow 6) and your left hand on the child's knees (Arrow 7). Do not wrap your hands around the knees or feet (ankles) or squeeze them together. Make sure the child's legs are straight.

8. **Measurer**: Ask the child to look straight ahead at the parent if she is kneeling in front of the child. Make sure the child's line of sight is parallel to the ground (Arrow 8). Place the thumb and index finger of your left hand, one finger on each side of the child's chin, and gradually close your hand (Arrow 9). Note that with most preschool-age children who are not heavy or obese, the back of the head will touch the back of the height board (Arrow 10); however, if the child is heavy or obese, there will be a space between the back of the child's head and the back of the measuring board. Make sure the child's shoulders are level (Arrow 11), the hands are at the child's side (Arrow

12), and at least the child's buttocks touch the back of the measuring board. Note that with most preschool-age children who are not heavy or obese, the back of the head, the shoulder blades, the buttocks, the calves and heels will touch the back of the measuring board (Arrows 10, 13, 14, 15 & 5).

9. **Measurer and Assistant**: Check the position of the child (Arrows 1-15). Repeat any steps as necessary.

10. **Measurer**: When the child's position is correct, lower the headpiece on top of the child's head (Arrow 16) making sure to push through the child's hair. Read and call out the measurement to the nearest 0.1 cm. Remove the headpiece from the child's head, your left hand from the child's chin, and allow the child to return to the parent.

11. **Assistant**: Immediately record the **height** measurements in **Questions 206** on the questionnaire and show it to the measurer. Record that the child was measured standing up in **Question 207.**

12. **Measurer**: Check the recorded measurement on the questionnaire for accuracy and legibility. Instruct the assistant to correct any errors.

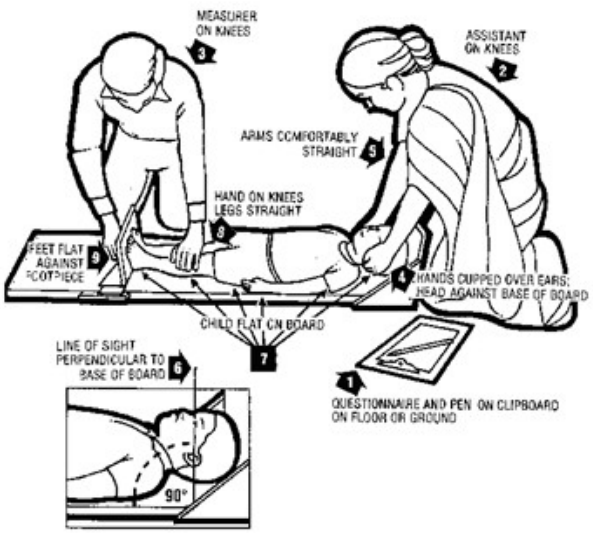
MEASURING A CHILD'S LENGTH (ILLUSTRATION 2)
for children less than two years old; or, when age cannot be obtained, length is measured for children less than 85 centimeters:

1. **Measurer or Assistant:** Place the measuring board on a hard, flat surface, such as the ground, floor or a solid table. Make sure the measuring board is stable.

2. **Assistant:** Place the questionnaire on the ground, floor or table (Arrow 1) and kneel behind the base of the measuring board if it is on the ground or floor (Arrow 2).

3. **Measurer:** Kneel at the right side of the child (at the child's feet) so that you can move the foot piece with your right hand (Arrow 3).

Illustration 2
Child Length Measurement



4. **Measurer and Assistant:** With the help of the parent, gently lower the child onto the measuring board, making sure the measurer supports the child at the trunk of the body while the assistant supports the child's head.

5. **Assistant:** Cup your hands over the child's ears (Arrow 4). With your arms straight (Arrow 5), place the child's head against the base of the board. The child should be looking straight up (Arrow 6) so that the line of sight is perpendicular to the board. Your head should be directly over the child's head. Watch the child's head to make sure it is in the correct position against the base of the board.

6. **Measurer:** Make sure the child is lying flat in the centre of the board (Arrow 7).

   Place the child's knees and feet in the correct position, with knees and feet either together or apart. There are three possible positions for the knees and feet:

   - Knees together and feet together
   - Knees together and feet apart
   - Knees apart and feet together

   } Whichever touches first!

   With your thumb against your index finger, place your left hand on the child's knees (Arrow 8) and press them gently, but firmly against the board. Do not wrap your hand around the knees or squeeze them together. Make sure the child's legs are straight.

7. **Measurer:** Check the position of the child (Arrows 1-8). Repeat any steps as necessary.

8. **Measurer:** When the child's position is correct, move the foot piece with your right hand until it is firmly against the child's heels (Arrow 9). Read the measurement to the nearest 0.1 cm and call out the measurement to the assistant. Return the child to the parent.

9. **Assistant:** Record the **height** measurements in **Questions 206** and that the child was measured lying down in **Question 207.** Show it to the assistant for confirmation.

10. **Measurer:** Check the recorded measurement on the questionnaire for accuracy and legibility. Instruct the assistant to correct any errors.

MEASURING AN ADULT'S HEIGHT (ILLUSTRATION 3):

NOTE: The height of adults can be taken by one person alone, the Measurer.

1. **Measurer:** Place the measuring board on a hard, flat surface against a wall, table, tree or staircase. Make sure the measuring board is stable. Many walls and floors are not at perfect right angles; if necessary, place small rocks underneath the height board to stabilize it during the measurement.

2. **Measurer:** Ask the person to take off his/her shoes and ask him/her to unbraid or push aside any hair that would interfere with the height measurement. Ask the person to stand on the base of the height measuring board and to face forward.

3. **Measurer:** Place the questionnaire and pen on the ground (Arrow 1) and stand on the left side of the person (Arrow 2).

4. **Measurer:** Determine if the person's feet should be against or away from the back of the height board by observing the imaginary line drawn from the tip of the shoulder to the heel, which is called the "mid-axillary line" (Arrow 3). This line should be perpendicular (90°) to the base of the height board where the person is standing. Note that with almost all adults you will have to move the person's feet away from the back of the height board to put them in the proper position; Arrow 4.

5. **Measurer:** Place the knees and feet in the correct position, with knees and feet either together or apart. There are three possible positions for the knees and feet:

    - Knees together and feet together
    - Knees together and feet apart
    - Knees apart and feet together

    } Whichever touches first!

6. **Measurer:** Ask the person to look straight ahead. Cup the respondent's chin between the thumb and index finger of your left hand and gradually close your hand (Arrow 5). Position the person's head so that the line of sight is parallel to the ground (Arrow 6). Note that with most adults, the back of the head will not touch the back of the height board—there will be a space between the back of the person's head and the back of the measuring board (Arrow 7). After you have placed the person's head in the proper position, release your hand from the person's chin and ask him/her to hold his/her head in the position you have just placed it in.

    Make sure the person's shoulders are level (Arrow 8), the hands are at the person's side (Arrow 9), and at least the buttocks touches the back of the measuring board). Note that with most adults, only the buttocks and perhaps the shoulder blades, will touch the back of the measuring board (Arrows 10 & 11).

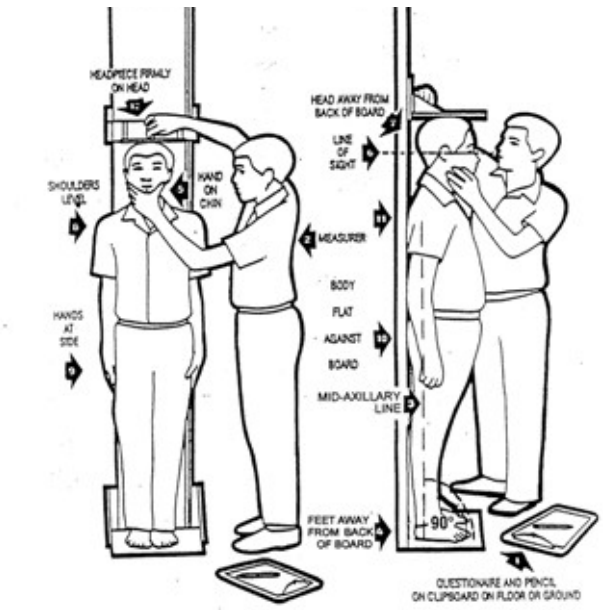
7. **Measurer:** Check the position of the person (Arrows 1-11). Repeat any steps as necessary.

8. **Measurer:** When the person's position is correct, lower the headpiece on top of the head (Arrow 12) making sure to push through the person's hair. Read and call out the measurement to the nearest 0.1 cm. Remove the headpiece from the person's head, and escort the person off the height board.

    Measurer: Immediately record the measurement

Standing Height of Adults*

Illustration 3



* From: "Anthropometry as Part of Household Surveys", I.J. Short, The World Bank, Washington, D.C., (in press)

on the questionnaire. Record an adult's **height** measurements in **Question 217/246**. If the adult's height was not measured, record the appropriate code in **Question 217/246**.

9. **Measurer:** Check the recorded measurement on the questionnaire for accuracy and legibility. Correct any errors.

Summary of Steps in Measuring Anthropometry

- Children less than 2 years old or, in absence of age information, less than 85 cm
  - Verify the recorded name and line number in Question 201 and date of birth in **Question 203**.
  - Confirm child was born in January [YEAR] or later
  - Measure weight
  - Record weight in **Question 205**
  - Measure length with child laying down on Shorr board
  - Record length in **Question 206**
  - Record that child was measured laying down in **Question 207**

- Children 2 years or older or, in absence of age information, 85 cm or greater
  - Verify the recorded name and line number in **Question 201** and date of birth in **Question 203**.
  - Confirm child was born in January [YEAR] or later
  - Measure weight
  - Record weight in **Question 205**
  - Measure standing height
  - Record height in Q206
  - Record that child was measured while standing in Q207

- Adults
  - Verify the recorded name and line number in Q215/244
  - Measure weight
  - Record weight in Q216/245
  - Measure height
  - Record height in Q217/246

CHAPTER 3: GENERAL PROCEDURES FOR COLLECTING CAPILLARY BLOOD DROP SAMPLES

Capillary blood will be collected in the [YEAR] [COUNTRY] DHS to test for the following biomarkers: [anemia] and [HIV]. Capillary blood can be obtained from the palm side of the end of a finger or from a heel. For adults and children six months of age and older, a finger should be used. However, if a child is undernourished (and skinny), the underlying tissue can be very thin and a lancet is likely to pierce the bone. For such children, a heel puncture is recommended. This chapter describes the materials needed for and, the steps involved in, capillary blood collection.

MATERIALS AND SUPPLIES FOR PERFORMING FINGER OR HEEL PRICK

The capillary blood drops collected for biomarker testing will be drawn from a finger or the heel. The following supplies and materials will be used in performing the finger or heel prick:

- **Disposable Latex gloves:** used to reduce the risk of bloodborne diseases. Gloves must be worn by the interviewer/health investigator and by anyone else who may assist with the blood collection.

- **Absorbent paper sheets:** the surface area where your supplies will be placed while you conduct the blood collection. Place the plastic/shiny side of the absorbent sheet down (the absorbent side without plastic on it should be up).

- **Alcohol preps:** used for cleaning the skin prior to pricking the finger or heel.

- **Safety lancets:** the lancet is a single-use, disposable device used to prick the fingertip or heel (Figure 3.1). The needle is retractable; when the trigger is pressed, a surgical blade quickly protrudes from the device, punctures the skin, and then automatically retracts. Lancets come in different sizes; one for use with children (depth of puncture is 2.25 mm) and one for use with adults (depth of puncture is 2.4 mm).

Figure 3.1 Examples of safety

- **Sterile gauze pads:** used to wipe away the first drop(s) of blood in order to stimulate a spontaneous capillary blood flow.

- **Adhesive bandages (plasters or Band-aids):** applied to the puncture site to minimize the risk of infection.

- **Plastic Bag for Waste:** Large bags that are provided to hold all of the biohazardous waste generated during the day. All waste bags are to be clearly labeled “biohazard”.

STEPS IN OBTAINING CAPILLARY BLOOD FROM THE FINGER

The following describes the steps that are involved in obtaining a capillary blood drop sample from the finger. They apply to both the collection of samples from adults and from children six months of age and older.

1. Complete general preparation

    - If possible, find an indoor site to encourage privacy. If possible, the site should have a table or other piece of furniture with a flat surface where you can lay out the supplies. A couch, bed or mat should be readily available if the respondent feels faint and needs to lie down.

    - If you find you must do the test outdoors, find a site in the full shade and away from rain, dust, and other environmental elements that might affect the sample.

    - When and where possible, wash and dry your hands. **Put on gloves** before beginning the collection of the blood sample from the first respondent.

    - Take out a clean **absorbent paper sheet** and spread it over a flat surface where you will lay out your supplies.

    - Refer to the Biomarker Data Form for children and adults to confirm the number of eligible individuals for whom blood samples will be collected. After you have established the number of individuals to test, take out the appropriate equipment and general supplies. You will want to have all general materials in easy reach when you begin collecting blood samples from the respondents.

        - **Note**- please do not remove microcuvettes or filter paper until right before pricking. These items should be taken out on an individual basis. In other words, if three individuals are being tested for anemia, only remove the microcuvettes from the canister one at a time, before pricking each person.

    - If the respondent is a child, describe to the parent/responsible adult exactly what will be done during the collection of the blood sample and how they can assist by holding the child on their lap and holding the child's hand during the collection of the sample.

    - The child may be fearful or anxious about what is going to happen. Therefore, using a calm and reassuring manner with the child is important as you begin to collect the blood sample. Remember that nonverbal communication is important, so maintain eye contact with the child as you prepare to take the sample.

2. Select and prepare the prick site

    - Blood collection is usually easier if you **sit on the side of the respondent opposite to the**

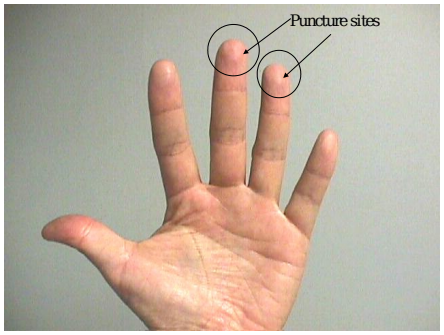


Figure 3.2 Fingers for blood

hand that you will collect blood from. For example, if you want to collect the specimen from the left hand, place yourself to the right side of the respondent.

- **Use the third or fourth finger** for collecting the blood (Figure 3.2). Do not use a finger with a scar, a wound or cut, an infection, swelling, a deformity, or a rash. Also, do not use a finger on which the respondent is wearing a ring, because the ring may disrupt the free flow of blood to the tip of the finger. You can ask the respondent to remove the ring.

- **Warm the skin** over the puncture site by rubbing it. This will increase blood flow to the finger tip and improve the ease with which a sample can be obtained.

- **With an alcohol swab, clean the skin of the finger** thoroughly (Figure 3.3). If the skin is very dirty, use a second swab. Finish cleaning the finger before preparing for the finger prick. Allow the alcohol to air dry. Do not blow on the area to dry the alcohol. Blowing may allow bacteria to contaminate the site.

Figure 3.3 Clean the finger

- **Ensure that the correct size lancet is easily accessible**. For <u>adults</u>, you will use adult lancets which have a needle diameter of 0.81 mm (21 G) and pierce the skin to a depth of <u>2.4 mm</u>. For children, you will use <u>children's lancets</u> which pierce the skin to a depth of <u>2.25 mm</u>.

- **Remove the blade slot cover**

  - For the child lancet, remove the blade slot cover by first twisting it 360º and then pulling it out.

  - For the adult lancets, push in the blade slot cover and then twist 360º. Pull it out after twisting.

  - ***Do not remove the blade slot cover from the adult or child lancets other than as instructed above,*** as this may cause the blade not to pierce the skin.

Figure 3.4 Parent assisting with

3. <u>Prick the Finger</u>

- **Make sure that the finger is below the level of the respondent's heart** to increase the flow of blood to the finger. Using a rolling movement of your thumb, **lightly press the finger from the top knuckle**

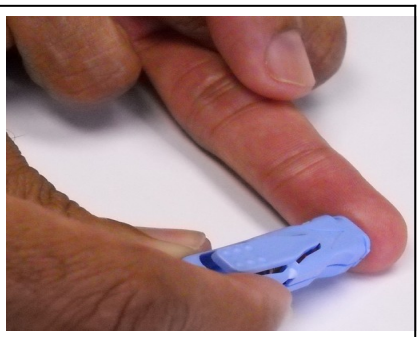


Figure 3.5 Placement of adult

toward the tip. This action will stimulate a flow of blood to the sample area.

- For children, it may be helpful if the parent/responsible adult assists you by holding the child's hand (Figure 3.4).
- When your thumb reaches the fingertip, **maintain a gentle pressure to trap the blood in the finger tip.**
- Place the **adult lancet** firmly against the skin with **the trigger facing upwards, so that the arrow preceding the trigger is visible** (Figure 3.5).
- For children, use the **child lancet** as shown in Figure 3.6. Place the lancet so that the **wide body of the lancet faces up**.
- Note: for both adults and children avoid placing the lancet on the very tip of the finger or the sides beyond the palmar area or you will risk piercing the underlying bone. Proper puncture sites are shown in Figure 3.7.

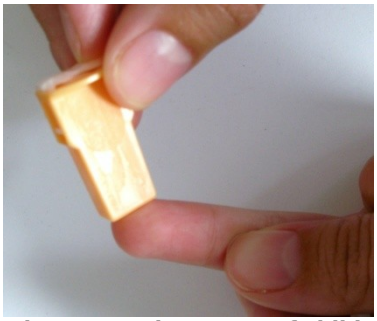


Figure 3.6 Placement of child

- Use the lancet to **prick the skin** by placing the blade-slot surface against the area and pressing the trigger (Figure 3.5 and 3.6). The tip of the blade ejects through the blade slot, producing a micro-incision in the skin, and immediately retracts into the device. After pricking the skin, set aside the lancet and turn the finger slightly to prevent blood from running into the grooves of the fingerprints.

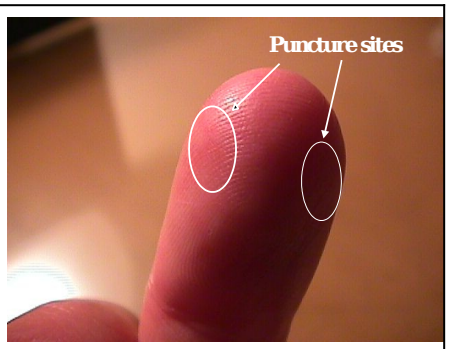


Figure 3.7 Puncture sites on the

4. Collect the blood drops

- When the blood appears, use a sterile gauze pad to wipe away *the first one or two drops of blood depending on the tests being performed*. [Note: anemia testing requires the first two drops be wiped away; blood collection for HIV testing requires only the first drop be wiped away (See Chapter 5 for more details on collecting blood for HIV). If blood is being obtained for both HIV and anemia testing, only the first drop is wiped away and blood collection for HIV testing precedes that for anemia testing. See Appendix B for more details on combining anemia and HIV blood collection.]
- If the blood stops flowing before you have collected at least three blood drops on the filter paper card and/or done the anemia testing, the pricking procedure may be repeated with the respondent's consent. For children or minors, you must get consent from the parent or

responsible adult. ***Do not reuse any of the supplies used for the first finger prick.***

5. Discard all materials used in the blood collection procedure in a labeled biohazardous waste container (bag).

STEPS IN OBTAINING CAPILLARY BLOOD FROM A CHILD'S HEEL

The ***heel*** is the puncture site for infants whose fingers are very thin. A child lancet of 2.25 mm will be used to puncture the heel. The following describes the steps that are involved in obtaining a capillary blood drop sample from the heel.

1. The prick should be made outside a line drawn from the middle of the big toe to the heel or outside a line drawn from the area between the fourth and fifth toes to the heel (Figure 3.8). Take care to avoid the central area of the foot (to avoid injury to the nerves and tendons) or the center of the heel (to avoid piercing the heel bone).

Figure 3.8 Site for heel puncture

2. Hold the heel firmly (Figure 3.9). Apply moderate pressure near the puncture site. This can be done by wrapping the heel using your thumb and second finger.

3. Clean the site with an alcohol swab. Make sure the site is dry before puncturing the skin with the lancet. In selecting a puncture site, avoid any areas of the skin that are broken or infected.

4. Use the lancet for the skin puncture by placing the blade-slot surface against the area and pressing the trigger. Ensure the free flow of blood.

Figure 3.9 Holding the infant's heel

5. Wipe away the first <u>two</u> drops of blood using a sterile gauze pad and collect the third drop for anemia testing (see Chapter 4).

6. Discard all materials used in the blood collection procedure in a labeled biohazardous waste container (bag).

PRECAUTIONS TO OBSERVE WHEN COLLECTING BLOOD SAMPLES[3]

This section describes the universal (general) precautions to be followed during blood collection[4]. You should take precautions when collecting blood to prevent exposure to bloodborne infections, such as hepatitis B, or human immunodeficiency virus (HIV). Under general precautions the following rules should be followed to ensure protection from acquiring bloodborne infections.

Wear gloves. Gloves help to prevent skin and mucous-membrane exposure to blood. Gloves should be worn during blood collection, until the specimen(s) from a respondent is collected and all waste materials produced during the collection are disposed. At that point, the gloves used should be treated as biohazardous waste. A new pair of gloves should be used with each respondent. **Gloves must never be reused! Avoid penetrating injuries.** Although gloves can prevent blood contamination of intact and non-intact skin surfaces, they cannot prevent penetrating injuries caused by the instruments used for finger or heel pricks. Safety lancet devices reduce the risk of penetrating injuries.

Lancets should not be used for purposes other than a single finger or heel prick to collect blood for the biomarker testing. The lancets should not be broken or destroyed for curiosity or other purposes. After the device is used, it should be placed in a puncture-resistant disposal biohazard bag.

If an accident occurs, any skin surfaces or mucous membranes that become contaminated with blood should be immediately and thoroughly washed with running water or copious amounts of standing water.

Never eat or drink during the testing. Since eating, drinking, and applying cosmetics may distract from the procedure, they are not permitted while collecting blood samples.

Properly dispose of all biohazardous materials. All materials coming in contact with blood must be placed in a biohazardous waste container after use and disposed of according to the survey's policy on infectious waste disposal (see Chapter 6). Take precautions when storing and transporting the waste container during the fieldwork.

[3] Adapted from National Committee for Clinical Laboratory Standards (NCCLS) 1997

[4] For the universal precautions regarding bloodborne pathogens, see the U.S. Centers for Disease Control and Prevention guidelines and the U.S. Occupational Safety and Health Administration (OSHA) standards for protection from exposure to bloodborne pathogen.

GOOD BLOOD COLLECTION PRACTICES

Good position in relation to the respondent. Position yourself well before you make a puncture on the respondent's finger.

Do not prick the finger if the hand is cold! Warm the hands by asking the respondent to rub them together vigorously. In the case of a child, ask the mother to rub the child's hands.

Never "milk" the finger. Excessive massaging or squeezing of the finger or foot will cause tissue juice to mix with and dilute the blood. This will result in erroneous test results, particularly yielding low levels of hemoglobin concentration in the blood. Instead, the tester should employ only mild pressure by using the thumb and the second and third fingers to make a "pad" at the puncture site. This will make the connective tissue underlying the skin more porous and allow the capillary blood to flow easily after the incision.

Never mix alcohol with the blood. If the alcohol used to clean the puncture site mixes with the blood, it can cause hemolysis of the sample leading to errors in the testing results. To avoid this problem, the finger or heel must be air dried completely before being punctured.

Avoid obstructing blood flow. It is important to hold the finger properly to allow for the accumulation of blood in the puncture-site area. Holding the finger too tightly can obstruct the blood flow to the finger.

Avoid shallow punctures. A deep puncture should be made for better blood flow and to have a representative concentration of red blood cells.

Dispose of biohazard materials as they are used. Keep the biohazard bag open during blood collection and drop each disposable item into the bag as you finish using it.

Summary of Steps for Capillary Blood Collection

- Seek informed consent
- For children between 6 and 12 months, determine if heel or finger prick should be performed
- Set up capillary blood collection supplies, making sure to select the correct lancet (adult or child)
- Clean surface of finger/heel
- Maintain gentle pressure to trap blood in the fingertip
- Apply moderate pressure near the puncture site of the heel
- Prick finger/heel
- Collect capillary blood
- Dispose of all testing materials in a clearly labeled biohazard bag or container.

CHAPTER 4: ANEMIA TESTING

Red blood cells contain hemoglobin (Hb), an iron-rich protein that binds oxygen in the lungs and carries it to tissues and organs throughout the body. Anemia is defined as a reduction in the normal number of red blood cells or a decrease in the concentration of Hb in the blood. Symptoms of anemia range from pallor, fatigue and weakness, shortness of breath and heart problems. During the [COUNTRY] DHS, we will measure the amount of Hb in the respondent's blood. Individuals who have an Hb level below a defined cut-off will be classified as anemic.

Common causes of anemia include:

- Iron deficiency from inadequate intake of foods containing iron, such as red meat;
- Intake of foods that contain non-bioavailable iron;
- Malaria and other parasitic infections (for example, schistosomiasis; hookworm);
- Blood disorders (for example, sickle cell anemia; thalassemia).

Anemia is a common and significant global health problem. Consequences of anemia include an increased risk of maternal and child mortality, impaired cognitive development in children, increased numbers of pre-term and low birthweight babies, and reduced work productivity in adults.

The measurement of Hb is the primary method of screening for anemia. Hb measurement provides an opportunity to:

- Estimate the prevalence of anemia in a nationally-representative sample;
- Link the levels of anemia with demographic data so as to examine the socioeconomic, residential, and demographic differences in the prevalence of anemia among populations;
- Design programs to prevent iron-deficiency anemia among the populations most in need of intervention (for example, iron fortification programs for women and young children living in rural districts).

Anemia testing in the [COUNTRY] DHS will be performed using a HemoCue photometer (Hb 201+). This widely used system measures Hb concentration from a drop of blood obtained from by finger prick. The test is rapid, allowing results to be reported to the respondent immediately following the testing procedure. Respondents found to have severe anemia will be referred to a health facility for treatment. Anemic status is considered severe with an Hb level below 9 g/dl for pregnant women, below 7 g/dl for women who are not pregnant or don't know if they are pregnant, below 9 g/dl for men and below 7 g/dl for children,
This chapter discusses the materials needed and the procedure for anemia testing. In addition, directions are given regarding precautions to take during collection and testing, recording results in the Biomarker Data Form, and providing test results and anemia information to respondents.

MATERIALS AND SUPPLIES FOR ANEMIA TESTING

In addition to the Biomarker Data Form and supplies listed in Chapter 3, the following equipment and supplies are required for anemia testing:

- **Microcuvette:** a plastic disposable unit that serves as both a reagent vessel and a measuring device (Figure 4.1). The tip of the microcuvette contains a dry, yellow reagent (sodium azide). The microcuvette is designed to draw up the exact amount of blood needed for the test.

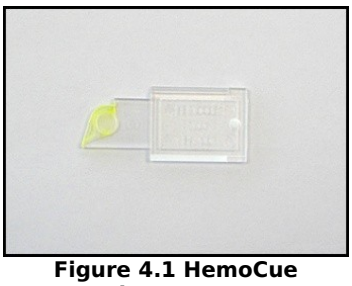


Figure 4.1 HemoCue

- **HemoCue Hb 201 + photometer**: a device that uses the absorption of light to measure hemoglobin concentration from a single drop of blood collected in a microcuvette (Figure 4.2). Test results are presented on the photometer's electronic display. The HemoCue system is described in greater detail below.

Figure 4.2 HemoCue Hb201+ photometer

- **Anemia Brochure:** a one page document designed to educate the respondent about anemia, including its definition, symptoms, causes, and methods of treatment and prevention. In addition, the respondents Hb results are recorded and classified within this document. See Appendix A for an example of an anemia brochure.

- **Anemia Referral Slip for Severely Anemic Respondents:** on this form is written the name and Hb results of severely anemic respondents. It is to be given to individuals with an Hb result indicative of severe anemia. They can take the referral to a local clinic or health center to receive proper treatment for their anemia.

<u>The HemoCue Photometer System (Hb 201+)</u>

Although the HemoCue system has proven to be durable and reliable under field conditions, there are some technical limitations related to the fact that microcuvettes are sensitive to humidity. Follow these instructions for the proper handling and storage of microcuvettes:

1. Record on the microcuvette container the date the container is first opened;
2. Remove from the container only those microcuvettes required for immediate testing;
3. Remove the microcuvettes by holding the side opposite the tip;
4. Immediately after taking a microcuvette out of the container, snap the container lid back on tightly;
5. Keep the microcuvette container at room temperature and avoid exposing it to heat or strong sunlight.

Under these conditions, a microcuvette container can be stored for up to 3 months (90 days) after opening. Under field conditions, it is advisable to store the microcuvettes in the opened container for no more than a month. Microcuvettes from unopened containers can be used up to the expiration date on the container.

To ensure the HemoCue Hb 201+ system operates properly, allow the photometer to come to the ambient temperature and protect it from direct sunlight. The device operates optimally between 18 and 30° C. The photometer has an internal electronic "SELFTEST"; every time the device is turned on, it automatically verifies the performance of its optronic unit.

The photometer's black microcuvette holder has three operating positions: 1) pushed in, for measuring; 2) pulled out until "clicked," for placing the microcuvette; 3) completely withdrawn for cleaning.

Clean the microcuvette holder (the drawer) at the end of each day's fieldwork. For cleaning the holder, use an alcohol swab or cotton wool/cotton-tipped swabs moistened with 70% alcohol. Follow these procedures to clean the microcuvette holder:

1. Check that the analyzer is turned off and the display window is blank.

2. Pull the microcuvette holder out of its loading position. Carefully press the small catch positioned in the upper right corner of the microcuvette holder.

3. While pressing the catch, carefully rotate the microcuvette holder towards the left as far as possible. Carefully pull the microcuvette holder away from the analyzer.

Figure 4.3 Cleaning microcuvette

4. Clean the microcuvette holder with an alcohol swab or cotton wool moistened with 70% alcohol (ethanol or isopropyl alcohol).

5. The HemoCue cleaner swabs are used to clean the optronic unit inside the machine. Clean the optronic unit by pushing the swab into the opening of the microcuvette holder (Figure 4.3). Move the cleaner from side to side 5-10 times. If the swab is stained (blood or dirt), repeat the cleaning procedure with a new swab. It is important that the microcuvette holder is completely dry prior to reinserting it in the photometer.

Blood may get on the optronic system if you do not wipe the outside of the microcuvette before placing the microcuvette in the holder. If this happens, you will get an error message (E01-E05; E09-E30). Clean the HemoCue machine as described above when you get an error message.

COLLECTING BLOOD AND TESTING FOR ANEMIA

Children: Follow the procedure below to test children for anemia:

1. Determine if the child is 6 or more months old and eligible for anemia testing: For **Question 208**, refer to **Question 203** to determine if the child is less than 6 months old If the child was born in the month of interview or in the 5 months before the month of interview, the child is not eligible for anemia testing. For example, if you are visiting the household in July, a child born in February, March, April, May, June, or July will not be eligible for testing. In other words, if the child is less than 6 months old, the child is not eligible for anemia testing, so you will record '1' and continue to the next child. If the child is older than 6 months, record '2' and proceed to **Question 209**.

2. Refer to the household schedule and record the **Line Number** of the parent/responsible adult of the child in **Question 209**.

3. Read the statement in **Question 210** to seek consent for anemia testing of the child from the parent/responsible adult. Record the outcome of the consent process in **Question 211.** Confirm that you read the statement to the parent/responsible adult and recorded their response accurately by signing in the space provided.

4. If consent was granted, collect a finger or heel stick blood sample from the child, following the procedure described in Chapter 3. Use a sterile gauze pad to wipe away the first two blood drops from the finger (heel) prick.

5. Conduct the anemia test as follows:

    - Step 1: Collect the capillary blood in the microcuvette:

        - Apply the tip of the HemoCue microcuvette to the middle of the blood drop. The microcuvette chamber will fill itself automatically by capillary action. The chamber needs to be filled completely (Figure 4.4). Never "top off" the microcuvette. Instead, if the microcuvette is not completely filled, use a fresh microcuvette and fill it with the next blood drop that forms.

        - Wipe any surplus blood off both sides of the microcuvette "like butter from a knife," using the clean end of a sterile gauze pad. Ensure that no blood is sucked out of the microcuvette when

Finger prick

Heel prick

Figure 4.4 Filling the microcuvette

wiping it – do not let the tip of the filled microcuvette touch the gauze.

- After filling the chamber, the microcuvette needs to be visually inspected for air bubbles. Since air bubbles may influence the hemoglobin measurement, any microcuvette containing air bubbles must be discarded. In such cases, with the permission of the parent/ responsible adult, repeat the blood drop collection using a different finger (heel). Again, you must use new disposable supplies and follow all of the steps described previously in obtaining the new sample.

- Place the microcuvette in its holder and gently push the holder into the photometer (Figure 4.5).

Figure 4.5 Placing the microcuvette in photometer

o Step 2: Stop bleeding at the site of the prick:

- After the blood drop collection, wipe any remaining blood from the prick site with a sterile gauze pad. Press the gauze pad against the prick site until the blood flow has stopped completely.

- Take an adhesive bandage from its wrapper and apply it to the prick site (Figure 4.6). Advise the mother, especially when the child is a toddler, to watch carefully that child does not take off the bandage and put it in his/her mouth where the child may choke on it.

Figure 4.6 Applying adhesive

o Step 3: Obtain the hemoglobin level:

- <u>Reading the results:</u> The microcuvette should be analyzed immediately, and no later than ten minutes after being filled. The blood hemoglobin level in grams per deciliter (g/dl) is displayed 15 to 45 seconds after the drawer is closed (Figure 4.6).

o Step 4: Record the hemoglobin level and test result:

- Record the hemoglobin level shown on the photometer (Figure 4.7) in the appropriate box in **Question 212** of the Biomarker Data Form. If there is

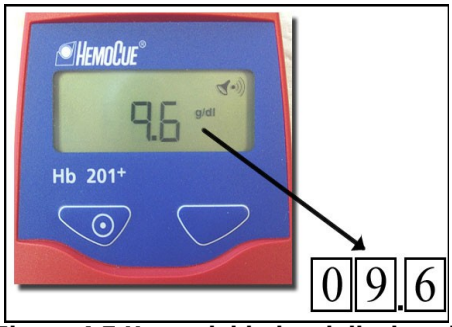


Figure 4.7 Hemoglobin level displayed on the HemoCue photometer and as it should be recorded on the Household

no value to record because the child was not present, the parent/responsible adult refused to consent to the test, or there was some other problem, record the appropriate code in **Question 212**. If more than one child in the household is eligible and is listed, check carefully that you are recording the hemoglobin level in the correct column of the schedule.

- Step 5: Collect biohazardous waste:
  - Place all biohazardous waste (lancets, microcuvettes, alcohol swabs, gauze, and gloves) into a plastic bag provided for field disposal of these items. At the end of the day, follow the procedures described in Chapter 6 for the proper disposal of waste materials.

6. Record the child's hemoglobin level in the anemia brochure. Inform the parent/responsible adult of the results and provide the parent with the brochure (see Appendix A).

7. Provide a written referral to a health facility for medical treatment for any child with severe anemia (hemoglobin level less than 7 g/dl; referral described at the end of chapter).

[WHEN PREPARING THIS MANUAL FOR A SPECIFIC SURVEY AND THE SURVEY CALLS FOR BOTH ANEMIA TESTING AND COLLECTING BLOOD FOR HIV TESTING:

Remove the 9 steps below for adult anemia testing (it will be covered in the "Combined procedure for anemia testing and collecting DBS for HIV testing".]

Adults: Follow the procedure below to test adults for anemia:

1. Check the respondent's age and marital status in **Question 218/247** and **Question 219/248** by referring to **Column 7** and **Column 8**, respectively, of the household schedule. Record whether a female respondent is age 15-17 or age 18-49; record whether the male respondent is age 15-17 or age 18-49. You must do this step because if the respondent is age 15-17 and never in a union, he/she is considered an adolescent and consent for testing must be obtained from the parent or responsible adult as well as the respondent. If the respondent has been in a union or is age 18-49, skip to **Question 223/252.**

2. **For a respondent age 18-49 or a respondent age 15-17 who has been in a union:**

    a. Seek consent for **anemia** testing from adult respondent in **Question 223/252** by reading the consent statement. If the adult does not consent to the anemia test, in **Question 224/253** record REFUSED, sign your name on the blank line. If the adult does consent, in **Question 224/253** record GRANTED, sign your name.

    b. For a female respondent who has consented to anemia testing, record pregnancy status in **Question 225**.

3. **For a respondent age 15-17 who has never been in a union (an adolescent):**

    a. Record the line number of his/her parent/responsible adult in **Question 220/249**.

    b. Seek consent for **anemia** testing from the parent/responsible adult in **Question 221/250**. If the parent/responsible adult does not consent to the adolescent's anemia test, in **Question 222/251** record REFUSED, sign your name on the blank line. If the parent/responsible adult does consent, in **Question 222/251** record GRANTED, sign your name, and go to **Question 223/252**.

    c. Seek consent for **anemia** testing from adolescent in **Question 223/252** by reading the consent statement. If the adolescent does not consent to the anemia test, in **Question 224/253** record REFUSED, sign your name on the blank line. If the adolescent does consent, in **Question 224/253** record GRANTED, sign your name.

    d. For a female respondent who has consented to anemia testing, record pregnancy status in **Question 225**.

4. Prepare the equipment and supplies for the tests for which consent has been granted as described in **Question 239/267**.

5. If consent was granted, collect blood from a finger prick following the procedure described in Chapter 3. Use a sterile gauze pad to wipe away the first two blood drops from the finger prick.

6. Conduct the anemia test as described above for children:

    - Step 1: Collect the capillary blood in the microcuvette
    - Step 2: Stop bleeding at the site of the prick
    - Step 3: Obtain the hemoglobin level
    - Step 4: Record the hemoglobin level and test result in **Question 240/268**.
    - Step 5: Collect biohazardous waste

7. Record the respondent's hemoglobin level in the anemia brochure.

8. Inform the respondent of her/his hemoglobin level and provide the anemia brochure.

9. Provide a written referral to a health facility for treatment for any respondent with severe anemia (below 9 g/dl for pregnant women, below 7 g/dl for women who are not pregnant (or don't know if they are pregnant), and below 9 g/dl for men.

PRECAUTIONS TO TAKE DURING ANEMIA TESTING

Please take the following precautions while doing anemia testing:

- **Never remove a microcuvette from the container with fingers wet with alcohol**. This can result in alcohol coming into contact with the reagents inside the microcuvette and destroying them. Using fingers wet with alcohol to handle other microcuvettes in the container can also affect them.

- **Never use the first two drops of blood for hemoglobin testing**. If the respondent consents to the anemia test, **wipe away the first two drops** of blood and then collect the third drop in the microcuvette. This ensures the free flow of blood and allows for the collection of blood with a representative concentration of red blood cells. [When testing for HIV in addition to anemia, follow the instructions provided in Appendix B, and use the fifth drop of blood for hemoglobin testing.]

- **Avoid inadequate filling or re-filling of the microcuvette**. The chamber of the microcuvette that contains dry reagents (yellow portion) has to be completely filled. The microcuvette should be filled with a drop of blood in one continuous motion. A microcuvette that contains air bubbles should be discarded.

- **Wiping off blood on the microcuvette**. Blood on the exterior of the microcuvette should be removed; failure to clean the exterior of the microcuvette can lead to an erroneously high hemoglobin reading.

- **Avoid keeping the microcuvette out for too long.** Keeping the microcuvette out of the container for too long before using it can lead to errors. Remove the microcuvettes from its container immediately before starting the testing procedure.

- **Avoid misalignment of the microcuvette in the photometer**. The microcuvette only fits into the photometer's microcuvette holder in one position. Therefore place it carefully in the holder and slowly push the holder inside the photometer to obtain a reading. Slamming the microcuvette holder can cause blood to spray onto the optronic system, an action that can damage the photometer.

- **Old or improperly stored microcuvettes should not be used for testing**. While in the field, microcuvettes should not be used if more than 1 month has elapsed since the seal on the container was broken. The containers must be kept closed when not in use to avoid exposure to moisture, which can destroy the reagents.

PROVIDING ANEMIA TEST RESULTS AND REFERRALS FOR SEVERE ANEMIA

Before leaving the household, you will verbally report the results of the hemoglobin measurement for each person for whom an anemia test was completed. In addition to verbally reporting Hb results, Hb results will also be written in an informational brochure that will be left at the household. When reporting the results, briefly explain to the respondent what his/her hemoglobin reading means, using the anemia brochure as a guide. Please see Appendix A for an example of the anemia brochure.

Respondents with severe anemia should be informed about the effects of severe anemia and recommended to visit a health facility for follow-up medical attention. For each respondent with severe anemia, you will fill out an Anemia Referral Slip (Figure 4.8), on which you have recorded the hemoglobin level.

FIGURE 4.8 EXAMPLE OF AN ANEMIA REFERRAL SLIP*

[COUNTRY] DEMOGRAPHIC AND HEALTH SURVEY

During the [COUNTRY] DHS, [YEAR], Ms./Mr./child_____
age __ __ years, was tested for anemia on __ __/__ __/__ __. His/her level of hemoglobin was __ __.__ g/dl, which indicates he/she is severely anemic. This person needs medical attention to treat the anemia

** You will use a referral slip designed by [IMPLEMENTING AGENCY].*

Summary of the steps involved in testing for anemia:

- Seek informed consent
- Clean the finger or heel with an alcohol swab;
- Prick the finger or heel with the appropriate-sized lancet;
  - Children 2.25 mm depth
  - Adults 2.4 mm depth
- Wipe away the first two drops of blood;
- Collect the third blood drop in a microcuvette;
- Stop the bleeding and apply an adhesive bandage to the puncture site;
- Measure hemoglobin level in the blood sample using the HemoCue photometer;
- Record the hemoglobin level in the appropriate column of the Biomarker Data Form;
- Collect biohazardous waste;
- Inform the respondent of his/her hemoglobin level and provide an informational brochure on anemia;
- Provide a written referral for follow-up medical attention for a respondent found to be severely anemic.

CHAPTER 5: DBS COLLECTION FOR HIV TESTING

Acquired Immune Deficiency syndrome (AIDS) is a disorder of the immune system caused by the human immunodeficiency virus (HIV). People with AIDS are vulnerable to contracting life-threatening infections that people with a properly functioning immune system would not typically develop.

Most of the current information on HIV prevalence in [COUNTRY] comes from surveillance of women attending antenatal clinics or of other special populations such as sex workers or individuals treated at health facilities for sexually transmitted infections. However, surveillance data do not yield an estimate of the prevalence of HIV among the general population. For example, the antenatal surveillance system excludes men and non-pregnant women. Since HIV is transmitted principally through sexual contact, obtaining an estimate of HIV prevalence for both men and women will provide a better estimate of the current level of HIV in [COUNTRY] than is available from other sources. Therefore, to estimate the national prevalence of HIV, for the [COUNTRY] DHS, a nationally-representative sample of women 15-49 and men 15-[49] will be tested.

HIV testing itself does not take place in the field. Instead, blood samples are collected from a finger prick on a filter paper card, dried, transported to the [IMPLEMENTING AGENCY] for logging in and checking, and brought to [NAME OF LAB] in [LOCATION]. The dried blood spots (DBS) will be stored at the laboratory until field work is completed and then processed for testing in batches.

HIV testing in the [COUNTRY] DHS is anonymous. Personal identifiers are delinked from the DBS-containing filter paper card, and respondents who agree to be tested are not told their results. Instead, respondents are given a voucher for voluntary counseling and HIV testing (VCT) at a nearby facility. For individuals living in communities in which there is no facility with the capacity to provide VCT within [15] km distance, arrangements will be made to bring mobile VCT to the community.

This chapter will describe the procedure for DBS collection for HIV testing. It includes:

- Required Materials and Supplies
- DBS Collection Procedure
- Collection Precautions
- Providing VCT Information
- DBS Storage and Transfer

MATERIALS AND SUPPLIES FOR DBS COLLECTION FOR HIV TESTING

In addition to the Biomarker Data Form and supplies listed in Chapter 3, the following materials are required for DBS collection for HIV testing:

- **Filter paper card**: You will use special filter paper cards (Figure 5.1) to collect the blood samples. Each card has five preprinted circles that hold about 100 µl of blood when filled.

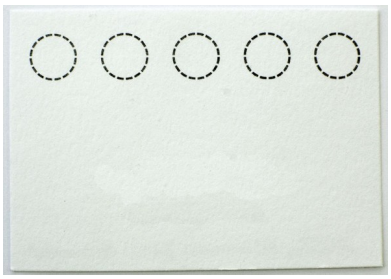


Figure 5.1 Filter paper card

    - **The pre-printed circles of the filter paper cards must be kept clean and dry at all times.** Water, dust, sweat from your hands, or other environmental contaminants can affect HIV testing. **Use gloves at all times when handling the filter paper cards.**

    - The filter paper cards come in 100 card packets. Before opening a new packet, put on gloves. Open the packet and place the cards in 3-4 smaller zip-loc bags; place these smaller bags in a large zip-loc bag. The bags must be stored stacked so as to avoid compressing the filter paper. **Place a few desiccant packets and a humidity indicator card in each small bag before sealing it**. **You must also place desiccants and a humidity indicator card in the larger zip-loc bag.** If the humidity indicator card and/or desiccants change color before the cards have all been used, replace the humidity indicator card and desiccants following the instructions described in the **Desiccant packets** and **Humidity indicator card** sections below.

    - Note: Keeping the unused filter paper cards with desiccants in a zip-loc bag will prevent moisture from being absorbed on the filter paper card which will prevent over-saturation or merging of circles when blood is collected.

- **Bar code labels:** because the HIV testing in the [COUNTRY] DHS is anonymous, respondent names are never written on the filter paper cards. Instead, bar code labels are used to identify the DBS samples and link them to the interview data. You will be provided with sheets of “peel-off” adhesive bar code labels (Figure 5.2). The barcodes are arranged in rows; the codes on each label are the same across one row. **A different row of bar code labels is to be used for each respondent for whom a DBS sample is collected.**

Figure 5.2 Bar code labels (sheet and individual).

- **Drying box:** a plastic box with a cardboard rack positioned inside.

- For proper use, stand the box vertically (the filter paper cards will be positioned horizontally). The drying boxes are to be used for overnight drying of DBS samples. They are not to be used for long-term sample storage.

- **Desiccant packets:** drying agents that absorb moisture from the air that are used to keep the filter paper cards as dry as possible (Figure 5.3). The granules inside the packets change color from blue to pink as they absorb moisture.
  - Change the desiccants when the granules change to a pink color or as indicated by the humidity indicator cards.
  - Treat used desiccants as biohazardous waste and throw them away in a biohazardous waste bag.

Figure 5.3 Desiccants

- **Humidity indicator cards:** cards that allow closer monitoring of the level of moisture than monitoring the color of the desiccant packets alone.
  - There are three circles on the humidity indicator card (Figure 5.4). If the circle at the bottom of the card (labeled 30%) turns pink, it indicates a relatively high level of humidity and is a warning to carefully monitor the humidity level. If the middle circle (labeled 40%) turns pink, replace the desiccant packets and humidity card. If the top circle (50%) turns pink, you must examine the DBS cards as their quality might have been compromised due to the high humidity in the bag. You should replace the desiccant packets and humidity card with fresh ones.

Figure 5.4 Humidity indicator

- **Glassine paper:** thin, glossy, semi-opaque paper squares used to protect the dried blood spots on the filter paper cards during storage.

- **Low gas-permeable bags (small zip-loc bags):** special small zip-loc bags used for storing the DBS samples in the field. These bags are specially manufactured to reduce the exposure of their contents to air and moisture. These bags are expensive and should never be used for other purposes, such as carrying food or adhesive bandages. The bags have a sliding “zipper” that is used to close and seal the bag.

- **Large zip-loc bags:** A large zip-loc bag will be provided for each of the [COUNTRY] DHS sample clusters in which you will work. These bags will be used to hold the small zip-loc bags with DBS samples from the cluster during storage and transport to the [IMPLEMENTING AGENCY] and [LABORATORY].

- **DBS Transmittal Sheet:** accompanies the DBS samples to the laboratory. The purpose of this form is to ensure that the number of DBS samples sent to the laboratory matches the number of samples collected in the field, and to track the samples throughout the transport process. A bar code with the same unique identifier as the bar code label attached to the DBS sample is also attached to the DBS Transmittal Sheet and to the Biomarker Data Form (Question 241 for women or Question 269 for men). See Appendix C for an example of the DBS Transmittal Sheet.

- **Voucher/Coupon for free VCT (Voluntary Counseling and HIV Testing) Services:** contains information regarding HIV, VCT services, and a list of local VCT centers where respondents can receive HIV testing. See Appendix D for an example of a Voucher/Coupon for free VCT Services.

[WHEN PREPARING THIS MANUAL FOR A SPECIFIC SURVEY AND THE SURVEY CALLS FOR BOTH ANEMIA TESTING AND COLLECTING BLOOD FOR HIV TESTING, FOLLOW THESE STEPS:

1) Replace section titled "DBS COLLECTION PROCEDURE" with APPENDIX B "Combined procedure for anemia testing and collecting DBS for HIV testing".
2) Keep the sections in this chapter titled "Precautions to take during DBS collection for HIV testing" and "Storing and transferring the DBS".
3) Replace the "Summary Steps" at the end of this chapter with the "Summary Steps" from Appendix B.
4) Change this chapter title to "ANEMIA TESTING AND DBS COLLECTION FOR HIV TESTING" and change the figure numbers in the remainder of this chapter.
5) Remove instructions for adult anemia testing found in chapter 4.

If combined testing is not part of the survey, remove Appendix B.]

DBS COLLECTION PROCEDURE

1. Identify all women and men from the household schedule who are eligible for DBS collection. Women age 15-49 and men 15-[49] years who are usual members of the household or who slept in the household the night before are eligible.

2. Record the **Line Number** and **Name** of all eligible women and men in **Question 215** and **Question 244**, respectively, of the **Biomarker Data Form.**

3. Check the respondent's age and marital status in **Question 218/247** and **Question 219/248** by referring to **Column 7** and **Column 8**, respectively, of the household schedule. Record the age group. You must do this step because if the respondent is age 15-17 and never in a union, he/she is considered to be an adolescent and consent for testing must be obtained from the parent or responsible adult as well as the respondent. If the respondent is or has been in a union or is age 18-49, skip to **Question 230/258.**

4. **<u>For a respondent age 18-49 or a respondent age 15-17 who has been in a union:</u>**

    - Read the informed consent statement for HIV testing to the respondent in **Question 230/258**. Record the outcome of the consent request in **Question 231/259**; confirm that you read the statement to the respondent and recorded their response accurately by signing in the space provided and entering your interviewer number.

    - Read the informed consent statement for **additional testing** to the respondent in **Question 236/264**. Record the outcome of the consent request in **Question 237/265**; confirm that you read the statement to the respondent and recorded their response accurately by signing in the space provided.

- If the respondent did not grant consent for additional testing, write **“No additional tests”** on the filter paper card. Go to **Question 239/267**.

5. **For a respondent age 15-17 who has never been in a union (an adolescent):**

   - Seek consent for **HIV testing** from the parent/responsible adult by reading **Question 228/256**. If the parent/responsible adult does not consent to the HIV test, record REFUSED in **Question 229/257**, sign your name on the blank line. If the parent/responsible adult does consent, in **Question 229/257**, record GRANTED and sign your name.

   - Read the informed consent statement for HIV testing to the respondent in **Question 230/258**. Record the outcome of the consent request in **Question 231/259**; confirm that you read the statement to the adolescent respondent and recorded their response accurately by signing in the space provided and entering your interviewer number.

   - Seek consent to store blood for **additional testing** from the parent/responsible adult by reading **Question 234/262**. If the parent or responsible adult does not consent to additional testing, record REFUSED in **Question 235/263**, sign your name on the blank line, and go to **Question 238/266**. If the parent/responsible adult consents, record GRANTED in **Question 235/263** and sign your name.

   - Read the informed consent statement for **additional testing** to the adolescent respondent in **Question 236/264**. Record the outcome of the consent request in **Question 237/265**; confirm that you read the statement to the adolescent respondent and recorded their response accurately by signing in the space provided.

   - If either the parent/responsible adult or the adolescent respondent does not grant consent for additional testing, write **“No additional tests”** on filter paper card.

6. If consent for HIV testing was granted, identify the next available complete set (row) of bar code labels. **Wearing a pair of latex gloves**, carefully remove a new filter paper card from the plastic zip-loc bag in which you have stored the cards. Make sure to handle the card in such a way that you do not touch the areas within the preprinted circle. *Never handle a card with your bare hands as you may transfer sweat, dirt or other contaminants on to the card*.

   Place the card with the pre-printed circles face-up on the clean absorbent sheet that you have spread out on a flat surface. Discard the card if it drops on the floor or ground or if it becomes dirty in any other manner.

- Take the **first bar code label** from the first complete **row** on the sheet of bar code labels and paste it in the column of the **Biomarker Data Form** containing the line number of ***this*** respondent (**Question 241** for women or **Question 269** for men).

- Take the **second bar code label** from the **same row** on the sheet of bar code labels and paste it at the bottom of the filter paper card where it says 'NAME'. Do not cover up or touch any part of the preprinted circles.

- Take the **third bar code label** from the ***same row*** on the sheet of bar code labels and paste it on the Blood Sample Transmittal Sheet for the cluster in which you are working.

- **If additional bar code labels will not be needed for a particular respondent, remove all extra bar code labels remaining in the row and discard them.**

- **DO THE ABOVE STEPS CAREFULLY**. The bar code label is the only means of identifying the blood sample and for linking the HIV test results to the interview data. Mistakes will result in mismatches later on. **CHECK THAT THE THREE MATCHING BAR CODE LABELS HAVE BEEN PLACED ON THE FILTER PAPER CARD, THE BIOMARKER DATA COLLECTION FORM, AND THE TRANSMITTAL SHEET BEFORE YOU PROCEED TO COLLECT BLOOD DROPS FROM THE RESPONDENT.**

7. Perform the DBS collection as follows:

   - Step 1: Obtaining Blood from the Finger

     - Follow the steps for producing a finger stick blood sample as described in Chapter 3. Use a sterile gauze pad to wipe away the first blood drop. While maintaining a firm grip on the finger, press gently on the side of the finger from which you are taking the blood sample to get a large second drop. Be careful to **avoid 'milking' or 'squeezing' the finger** as this could affect the test results. Wait until the drop is large enough to fill one of the preprinted circles on the card (Figure 5.5).

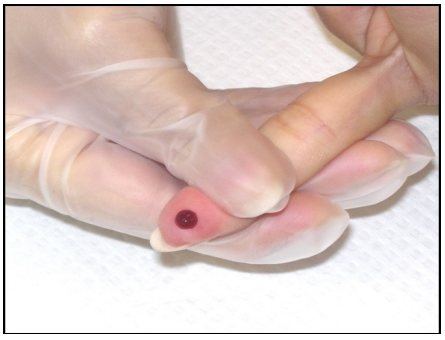


Figure 5.5 Large drop of blood

   - Step 2: Position of Filter Paper Card

     - Move the card underneath the finger, with the pre-printed side of the card facing the pricked finger.

     - The card must not be pressed against the prick site on the finger. Make sure that the respondent's finger does not

touch the card at any point when you are collecting the blood spots.

- Step 3: Blood Collection on the card:
  - Let the blood drop fall freely in the center of the preprinted circle. In case the blood drop does not fall readily, you may touch the filter paper gently against a LARGE blood drop (but not the skin). In one step, a sufficient quantity of blood should be allowed to soak through and completely fill the circle (Figure 5.6).

Figure 5.6 Filling up the pre-printed circle on the filter paper card

  - To enhance blood flow, gently apply intermittent pressure to the area surrounding the prick site to get a third drop. Allow sufficient time for a large blood drop to form before filling the next circle on the filter paper card. Again, avoid milking or squeezing the finger.
  - You must continue to collect drops of blood until you have fully saturated at least three circles on the filter paper card (Figure 5.7).
  - If possible, continue to fill the remaining circles with blood.
  - If the blood flow stops or decreases before you fully saturate the three circles, you will need to do another finger prick. Whenever this is necessary, you should explain to the respondent that you were unable to obtain an adequate sample and ask permission to obtain blood from another finger. Use fresh supplies and a **different finger** for the second finger prick.

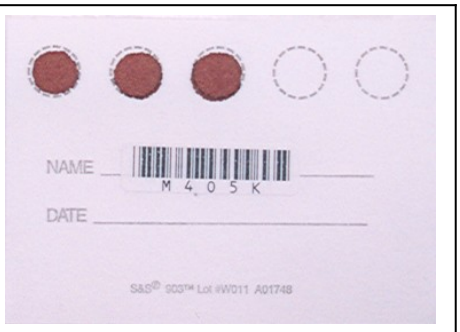


Figure 5.7 Fully-filled circles without layering or caking

  - Place the filter paper card with the blood spots on the absorbent paper sheet away from other items. Be careful not to drop the completed filter paper card.
- Step 4: Stopping bleeding at puncture site
  - After the blood drop collection, wipe any remaining blood from the puncture site with a sterile gauze pad. Press the

gauze pad against the prick site until the blood flow has completely stopped.

- Take an adhesive bandage from its wrapper and apply it to the prick site.

- Step 5: Placing the completed filter paper card in the drying box

  - The drying box should be placed vertically on a flat surface at the time you set up your equipment. The position of the drying box is especially important anytime it contains filter paper cards that have not completely dried. Keep the box vertical whenever it contains blood spots that are not fully dried to prevent blood that has not yet completely dried from spreading.

  - Keep the drying box closed during blood collection to prevent dust and dirt from entering the box or from contaminating DBS cards that were collected earlier and are already in the box. After the blood collection process is finished, open the drying box (keeping it vertical). Carefully pick up the completed filter paper card and place it in a horizontal position in one of the slots in the drying rack in the box. The blood spots should face towards the back of the drying box. Close the box.

  - ***Avoid touching or smearing the blood spots on other cards already in the box when you are storing a new card. Never put more than one completed filter paper card in a single slot in the drying rack.***

  - Allow blood spots to dry overnight at ambient temperature. The box should be handled carefully so that the cards do not fall out of their slots.

- Step 6: Collecting biohazardous waste

  - Place all biohazardous waste (lancets, alcohol swabs, gauze pads, and gloves) into a clearly labeled “biohazard” plastic bag, which has been provided for field disposal of these items. At the end of the day, follow the procedures described in Chapter 6 for the proper disposal of waste materials.

8. Record the result for blood collection for HIV testing in **Question 241/269.**

9. Provide an informational brochure on HIV/AIDS. The brochure will include a voucher for free HIV VCT services, and a list of the nearest participating VCT centers in [COUNTRY].

PRECAUTIONS TO TAKE DURING DBS COLLECTION FOR HIV TESTING

With respect to DBS collection for HIV testing, there are a number of precautions that should be carefully observed including:

- **Always use the pre-printed side of the card to collect the blood spots.**

- **Do not 'layer' the sample in an attempt to fill in the circle**.

    - There may be times when a drop of blood will not completely fill the circle. If a circle is not completely saturated, the next drop or just a portion of the next drop of blood may be used to saturate the circle if the drop is obtained immediately. If the first drop starts to dry due to any interruption in getting the subsequent drop, you must begin filling another circle. Layering or application of successive drops of blood to a dried or partially dried blood spot causes caking.

- **Do not overfill the circles**. Overfilling the circles can cause super saturation (top panel of Figure 5.8), which is unacceptable.

- **Do not place box horizontally until blood dries**. Placing the box horizontally before the blood dries can cause serum rings (bottom panel of Figure 5.8), which is also unacceptable. The blood should not extend beyond the pre-printed area, as shown in Figure 5.7.

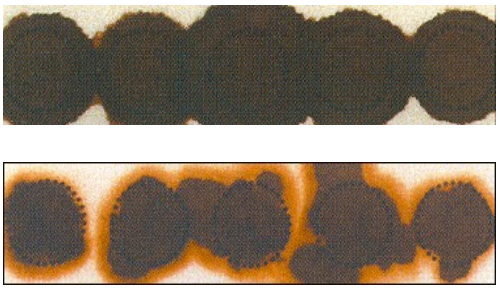


Figure 5.8 Supersaturated (top) and serum rings (bottom) DBS samples are

- Try to have the first drop fall exactly in the center of the pre-printed circle. However, if by accident the drop falls outside of the circle and is not large enough, then let the next drop of blood fall again exactly in the center of the original drop and not in the center of the pre-printed circle. Note: **all circles should have uniform blood volume.**

- **Protect the filter card from contamination**. Do not allow water or other contaminants to come into contact with the specimen card before or after use.

- **Do not place the specimens in the small zip-loc bags until the blood has dried thoroughly** (chocolate brown). Insufficient drying adversely affects the quality of the samples and consequently, the test results.

- **Taking the filter paper card out of the storage bag**. The filter paper card should be the last item taken out of the package before starting the blood collection procedure.

STORING AND TRANSFERRING THE DRIED BLOOD SPOTS

The dried blood spot (DBS) samples must be carefully maintained until they are picked up and taken to the laboratory. They should never be exposed to sunlight during storage, and it is important to regularly monitor the level of humidity in the stored samples. The following describes the steps that should be followed in storing and transferring the DBS samples.

Storage: Each morning, before you go to the field, you must remove the filter paper cards with the DBS samples that you collected on the previous day from the drying box and prepare them for storage as follows:

1. Put on a pair of latex gloves and carefully open the drying box. Check that the blood spots on each filter paper are completely dried (chocolate brown).

2. Separately remove each filter paper card on which the spots have dried from the drying box. Be careful not to touch the blood spots.

3. Gently fold a piece of glassine paper over the blood spots and put one filter paper card into a small (low gas-permeable) zip-loc bag. Put one desiccant packet and a humidity indicator card behind the filter paper card, with the circles on the humidity card and the 'window' in the desiccant sachet facing out so that the humidity indicator level and granules, respectively, are visible. It is important that the desiccant packet and the humidity indicator card do not touch the blood spots. Close the zipper, gently pushing out any excess air in the bag as you are zipping it, being careful not to press on the blood spots. **DBS samples should not be allowed to come into contact with other DBS samples during handling, shipment or storage.**

4. Continue to package each of the filter paper cards from the previous day which have dried overnight, putting one filter paper card into one small zip-loc bag with one desiccant packet and one humidity indicator card.

    - When you have packaged all of the filter paper cards, put them into the large zip-loc bag that has been labeled for the cluster in which the samples were collected (Figure 5.9). Note that the Cluster Sample (zip-loc) Bag itself should also contain a few desiccant packets.

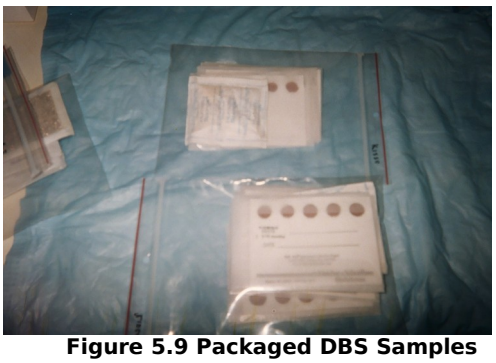


Figure 5.9 Packaged DBS Samples

5. Check the humidity indicator cards for the individually packaged DBS samples that you have previously placed in the Cluster bag before adding newly packaged DBS samples to an existing Cluster Sample Bag. **The buildup of humidity can damage the quality of the sample.**

    - A bottom circle that is pink (30% humidity) indicates a warning of increasing humidity. If the middle circle (40%) or top circle (50%) is

pink, gently open the small zip-loc bag, remove the desiccant packet and replace it with a fresh desiccant packet. If any of the circles on the humidity indicator card have merged together so that they are not completely separated, remove the indicator card and replace it with a fresh indicator card. Close the zipper, gently pushing out any excess air in the bag as you are zipping it. **Please review the storage instructions, using the humidity cards as a guide.**

6. Check the condition of the desiccant packets and humidity indicator card before closing the zipper on the Cluster Sample Bag. Replace as needed.

    - If you have additional Cluster Sample Bags for completed clusters that have not yet been collected by a Field Supervisor, examine all of the samples in those bags in the same manner, every couple of days, as long as they are with your team in the field.

Transfer: The purpose of the DBSTransmittal Form (see Appendix C) is to account for the samples at each step of the way.

1. Fold the DBS Transmittal Form along the dotted lines (so that the bar-code labels are not folded), and keep it in the Sample Cluster Bag along with the DBS samples for that cluster.

2. When you have completed the cluster, remove the packaged DBS samples from the Cluster Sample Bag (do not open the small zip-loc bags).

3. One by one, check the bar codes on the labels on the filter paper cards against the bar codes affixed to the back side of the DBS Transmittal Sheet. For each DBS sample, put a check mark in the column labeled TECHNICIAN for each corresponding bar code found on the transmittal sheet. Count the number of DBS samples and record in the boxes provided in Column (3) on the front side of the transmittal sheet in the column labeled TOTAL COUNT OF BLOOD SAMPLES. If there are any discrepancies, you must attempt to account for them. Use Column (7) to explain. Sign your name in Column (4) and the date in Column (6).

The team's field supervisor will follow behind you, re-verify the samples, and sign his/her name in the FIELD TEAM SUPERVISOR row.

Periodically, a sample pick up person/vehicle will visit the teams to collect the DBS samples for the completed clusters. When he/she collects the DBS samples, he/she will recount the DBS samples for each of the completed clusters and sign the DBS Transmittal Sheet. The samples and transmittal sheet will be transported to [IMPLEMENTING AGENCY] for logging before being transferred to the laboratory for processing.

[WHEN PREPARING THIS MANUAL FOR A SPECIFIC SURVEY AND THE SURVEY CALLS FOR COMBINED ANEMIA/HIV TESTING:

Replace the "Summary of the steps involved in collecting blood for HIV testing" below with the "Summary of the steps involved in collecting blood for HIV testing and for anemia testing" from APPENDIX B.]

Summary of the steps involved in collecting blood for HIV testing:

- Prepare Biomarker Data Form for individuals eligible for testing;
  - Seek voluntary consent for blood collection for HIV testing from the respondent (if the respondent is age 15 to 17 and unmarried seek voluntary consent from the parent/responsible adult and the respondent).
- Place a bar code label on a filter paper card, the Biomarker Data Form, and the DBS Transmittal Sheet;
- Clean and prick the respondent's finger with an adult lancet;
- Wipe away the first drop of blood;
- Fill the pre-printed circles on the filter paper card with the second, third, and fourth drops of blood;
- If possible, continue to collect the fifth and sixth blood drops on the filter paper;
- Collect the sixth and seventh blood drops on the filter paper, if possible;
- Stop the bleeding at the prick site;
- Place the filter paper card in the drying box;
- Collect biohazardous waste.
- Provide all respondents with an informational brochure on HIV/AIDS, vouchers for free VCT services, and a list of nearby VCT centers.

CHAPTER 6: BIOHAZARDOUS WASTE DISPOSAL

Any material coming in contact with blood or serum (lancets, microcuvettes, alcohol swabs, gauze, and gloves) is considered to be biohazardous (hazardous to other humans). Safe disposal of such material is very important to prevent the transmission and spread of various bloodborne diseases, such as hepatitis B and HIV, among survey personnel and within the study community. Biohazardous waste has to be collected in a special container during the blood collection and testing, securely stored and transported, and safely disposed at the end of each day of fieldwork.

If possible, commercially available biohazardous waste disposal containers should be used for waste disposal. These types of containers are red and have a special logo warning about biohazardous content. They can be securely closed for safe storage and transportation during the fieldwork.

There are two options for disposal:

1) Take the biohazardous waste to the nearest health facility for disposal in an incinerator **(preferred option)**. The health facilities should employ standard procedures for biohazardous waste disposal.
2) Follow the procedures outlined below for burning the waste in the field.

MATERIALS AND SUPPLIES

The following items are required in the field for disposal of biohazardous materials:

- Kerosene
- Four percent sodium hypochlorite solution[5]
- Matches
- Spade or other tool for digging a small pit
- Ziploc-type polyethylene bags
- Forceps
- Puncture-resistant container labeled "Biohazard" (for example, a wide-mouth plastic jar).
- Scissors

PROCEDURES FOR FIELD DISPOSAL OF BIOHAZARDOUS WASTE

At the end of each blood collection and hemoglobin measurement within the household, all materials used during the testing (gloves, microcuvettes, lancets, alcohol swabs, and gauze pads) are to be placed in a biohazard bag (plastic). Upon return to the field house/site, the waste is transferred to the sharps container (a wide-mouth plastic jar), which will be disposed of following the procedure below.

Before beginning the biohazardous waste disposal procedure, determine a place where the waste can be safely destroyed. An open field area with loose soil is preferable, since the materials need to be burnt and buried. To reduce the risk

[5] Four percent sodium hypochlorite solution could be purchased as a commercially available product. It could also be prepared in the field by substituting a hypochlorite powder using water. The liquid solutions (sodium hypochlorite solution and kerosene) should be stored in leak proof and airtight containers.

of spreading a fire, avoid starting a fire in drought areas, and keep away from other flammable materials.
Follow the procedure below to safely dispose of biohazardous waste in the field:

Step 1: At the end of each day, bring the sharps container (plastic jar) with biohazardous materials to the area selected for the waste disposal. Wearing gloves, add a half liter of 4 percent sodium hypochlorite solution into the sharps container (plastic jar) with the biohazardous materials (see figure 6.1). After adding the hypochlorite solution, close the container (jar) so it is airtight. Keep the jar in an upright position for five minutes. After that, invert the plastic jar and keep in that position for an additional five minutes. This step is necessary to ensure that all of the materials in the sharps container (plastic jar) are disinfected by complete immersion in the 4 percent sodium hypochlorite solution.

Figure 6.1 Adding sodium hypochlorite

Figure 6.2 Transferring contaminated materials

Step 2: Transfer the contents of the plastic jar, including the sodium hypochlorite solution to a thick polyethylene bag (Figure 6.2).

Step 3: A forceps can be used to transfer any material that sticks to the walls of the plastic jar to the polyethylene bag (Figure 6.3).

Figure 6.3 Removing remaining materials

Figure 6.4 Digging a pit for bag

Step 4: Dig a small hole with a spade, and put the polyethylene bag containing the biohazardous materials in the pit (Figure 6.4).

Step 5: Use scissors to make a hole at the bottom of the polyethylene bag (Figure 6.5).

Figure 6.5 Making a hole in the bag

Step 6: Drain off the hypochlorite solution from the polyethylene bag (Figure 6.6).

Figure 6.6 Draining off hypochlorite solution

Step 7: Put waste paper on top of the polyethylene bag containing biohazardous materials (Figure 6.7).

Figure 6.7 Putting waste paper on top of bag

Figure 6.8 Pouring kerosene on bag

Step 8: Pour kerosene on the bag (Figure 6.8).

Step 9: Burn the polyethylene bag containing the biohazardous materials in the pit (Figure 6.9).

Figure 6.9 Burning contaminated materials

Figure 6.10 Ascertaining that contaminated materials are completely burned

Step 10: Wait until all of the contents are burned (Figure 6.10).

Step 11: Cover the pit with soil (Figure 6.11).

Figure 6.11 Covering the pit with soil

It is your responsibility to ensure proper disposal of biohazardous waste. It is unacceptable that the materials used during the testing in one fieldwork cluster are carried by the team to the next cluster. Biohazardous materials must be destroyed at the end of the day.

APPENDIX A: Anemia Brochure

What is anemia?
Anemia is a serious health condition in which there are not enough red blood cells or hemoglobin in the blood.

Hemoglobin is a substance in the blood that carries oxygen to the brain, muscles, disease-fighting organs and other parts of the body. Iron is important for making hemoglobin.

What are the symptoms of anemia?
Some of the symptoms of anemia are:
- ◊ tiredness
- ◊ headaches
- ◊ dizziness
- ◊ poor appetite
- ◊ heart palpitations
- ◊ shortness of breath

Why is anemia dangerous?
Anemia is dangerous because:
- ◊ it reduces one's resistance to infections
- ◊ severe anemia can lead to heart failure
- ◊ during childbirth, anemic women are more likely to die from excessive bleeding
- ◊ anemic children have low birth weight, poor learning capacity, and less resistance to infections than other children

What causes anemia?
Anemia is caused by:
- ◊ loss of blood due to
  - ❖ malaria;
  - ❖ parasites, especially hookworms;
  - ❖ excessive menstrual losses;
  - ❖ chronic diseases such as ulcers or tuberculosis.
- ◊ lack of iron in the diet
- ◊ inability of the body to absorb iron from food

TEST RESULTS

Date _____

WEIGHT, HEIGHT, and HEMOGLOBIN MEASURES			
Name	Name	Name	Name
WEIGHT (kg)			
HEIGHT (cm)			
HEMOGLOBIN (g/dl)			

Hemoglobin level (grams per decilitre)				
Diagnosis	Severe Anemia	Moderate Anemia	Mild Anemia	Normal
Children <6 years	<7.0	7.0-9.9	10.0-10.9	11.0 +
Women not pregnant	<7.0	7.0-9.9	10.0-11.9	12.0 +
Pregnant women	<9.0	9.0-9.9	10.0-10.9	11.0 +
Men	<9.0	9.0-11.9	12.0-12.9	13.0 +

How can anemia be prevented?
- ◊ Pregnant mothers and infants should take iron tablets or syrup.
- ◊ Eat a diet adequate in iron-rich foods such as dark green vegetables, liver, meat or fish, and fruits rich in vitamin C.
- ◊ Avoid giving tea to infants and young children.
- ◊ Avoid taking coffee or tea with meals.
- ◊ Prevent and treat worms.
- ◊ Prevent malaria by using mosquito nets.
- ◊ Limit the number of births through child spacing and delaying first pregnancies.

What do the test results mean?
Severe anemia: You have a seriously low level of hemoglobin in your blood. You need to see your doctor or health center immediately for treatment. Eat more foods rich in iron and treat malaria and worms immediately.

Moderate anemia: You have a reduced level of hemoglobin. Iron deficiency, worms, excessive bleeding, or malaria may cause you to be anemic. You should visit your doctor or health center as soon as possible. Eat more foods rich in iron.

Mild anemia: Your hemoglobin level is slightly lower than normal. You need more daily iron. Treat malaria and worms immediately. Eat more foods rich in iron, such as dark green vegetables, meat, liver or fish.

To increase the body's use of iron, eat more fruits rich in vitamin C, such as oranges, lemons, mangoes.

APPENDIX B: COMBINED PROCEDURE FOR ANEMIA TESTING AND COLLECTING DBS FOR HIV TESTING

This section focuses on the steps involved in collecting the blood samples for both HIV and anemia testing. When blood is collected for both HIV and anemia testing, the order of collection is very important and must be followed strictly. Blood is to be collected for HIV first, while collection of blood for anemia testing follows.

Consent for each test must be separately obtained, following the order of the Biomarker Data Form. If a test has not been consented to, follow the skip instructions. **You should complete the testing process with each respondent before proceeding to the next eligible individual.**

Follow the procedure below to collect blood for both filter paper cards for HIV testing and anemia testing:

2. Identify all women and men from the household schedule who are eligible for anemia and DBS collection. Women age 15-49 and men 15-[49] years who are usual members of the household or who slept in the household the night before are eligible.

3. Record the **Line Number** and **Name** of all eligible women and men in **Question 215** and **Question 244**, respectively, on the **Biomarker Data Form.**

4. Check the respondent's age and marital status in **Question 218/247** and **Question 219/248** by referring to **Column 7** and **Column 8**, respectively, of the household schedule. Record the age group. You must do this step because if the respondent is age 15-17 and never in a union, he/she is considered to be an adolescent and consent for testing must be obtained from the parent or responsible adult as well as the respondent. If the respondent is in a union or has been in a union or is age 18-49, skip to **Question 223/252.**

5. **For a respondent age 18-49 or a respondent age 15-17 who has been in a union:**

    - Read the informed consent statement in **Question 223/252** to the respondent and record the outcome of the consent request in **Question 224/253**. If the respondent does not consent to anemia testing, follow skips to **Question 230/258**.

    - For a female respondent who has consented to anemia testing, record pregnancy status in **Question 225**.

    - Read the informed consent statement for HIV testing to the respondent in **Question 230/258**. Record the outcome of the consent request in **Question 231/259**; confirm that you read the statement to the respondent and recorded their response accurately by signing in the space provided and entering your interviewer number. Follow the skips to **Question 236/264.**

- Read the informed consent statement for **additional testing** to the respondent in **Question 236/264**. Record the outcome of the consent request in **Question 237/265**; confirm that you read the statement to the respondent and recorded their response accurately by signing in the space provided.

- If the respondent did not grant consent for additional testing, write **"No additional tests"** on the filter paper card. Go to **Question 239/267**.

6. **<u>For a respondent age 15-17 who has never been in a union (an adolescent)</u>:**

    - Record the line number of his/her parent/responsible adult in **Question 220/249**.

    - Seek consent for **anemia** testing from the parent/responsible adult in **Question 221/250**. If the parent/responsible adult does not consent to the adolescent's anemia test, in **Question 222/251** record REFUSED, sign your name on the blank line, and go to **Question 228/256**. If the parent/responsible adult does consent, in **Question 222/251** record GRANTED, sign your name, and go to **Question 223/252**.

    - Read the informed consent statement in **Question 223/252** to the adolescent respondent and record the outcome of the consent request in **Question 224/253**. If the adolescent respondent does not consent to anemia testing, skip to **Question 228/256**.

    - For a female adolescent respondent who has consented to anemia testing, record pregnancy status in **Question 225**.

    - Seek consent for **HIV testing** from the parent/responsible adult by reading **Question 228/256**. If the parent/responsible adult does not consent to the HIV test, record REFUSED in **Question 229/257**, sign your name on the blank line, and go to **Question 239/267**. If the parent/responsible adult does consent, in **Question 229/257**, record GRANTED and sign your name.

    - Read the informed consent statement for HIV testing to the adolescent respondent in **Question 230/258**. Record the outcome of the consent request in **Question 231/259**; confirm that you read the statement to the respondent and recorded their response accurately by signing in the space provided and entering your interviewer number.

    - Seek consent to store blood for **additional testing** from the parent/responsible adult by reading **Question 234/262**. If the parent or responsible adult does not consent to additional testing, record REFUSED in **Question 235/263**, sign your name on the blank line, and go to **Question 238/266**. If the parent/responsible adult consents, record GRANTED in **Question 235/263** and sign your name.

- Read the informed consent statement for **additional testing** to the adolescent respondent in **Question 236/264**. Record the outcome of the consent request in **Question 237/265**; confirm that you read the statement to the adolescent respondent and recorded their response accurately by signing in the space provided.

- If either the parent/responsible adult or the adolescent respondent does not grant consent for additional testing, write **"No additional tests"** on filter paper card.

7. Prepare the equipment and supplies for the tests for which consent has been granted as described in **Question 239/267**.

8. If consent for HIV testing was granted, identify the next available complete set (**row**) of bar code labels. **Wearing a pair of latex gloves**, carefully remove a new filter paper card from the plastic zip-loc bag in which you have stored the cards. Make sure to handle the card in such a way that you do not touch the areas within the preprinted circle. *Never handle a card with your bare hands as you may transfer sweat, dirt or other contaminants on to the card*.

    - Place the card with the preprinted circles face-up on the clean absorbent sheet that you have spread out, on a flat surface. Discard the card if it drops on the floor or ground or if it becomes dirty in any other manner.

    - Take the **first bar code label** from the first complete row on the sheet of bar code labels and paste it in the column of the Biomarker Data Form containing the line number of ***this*** respondent (**Question 241** for women or **Question 269** for men).

    - Take the **second bar code label** from the same row on the sheet of bar code labels and paste it at the bottom of the filter paper card where it says 'NAME'. Do not cover up or touch any part of the pre-printed circles.

    - Take the **third bar code label** from the ***same row*** on the sheet of bar code labels and paste it on the Blood Sample Transmittal Sheet for the cluster in which you are working.

    - **Remove all extra bar code labels remaining in the row and discard them if applicable.**

    - **DO THE ABOVE STEPS CAREFULLY**. The bar code label is the only means of identifying the blood sample and for linking the HIV test results to the interview data. Mistakes will result in mismatches later on. **CHECK THAT THE THREE MATCHING BAR CODE LABELS HAVE BEEN PLACED ON THE FILTER PAPER CARD, THE BIOMARKER DATA COLLECTION FORM, AND THE TRANSMITTAL SHEET BEFORE YOU PROCEED TO COLLECT BLOOD DROPS FROM THE RESPONDENT.**

9. Perform the DBS collection as follows:

- Step 1: Obtaining Blood from the Finger

  - Follow the steps for producing a finger stick blood sample as described in Chapter 3. Use a sterile gauze pad to wipe away the first blood drop. While maintaining a firm grip on the finger, press gently on the side of the finger from which you are taking the blood sample to get a large second drop. Be careful to **avoid 'milking' or 'squeezing' the finger** as this could affect the test results. Wait until the drop is large enough to fill one of the pre-printed circles on the card (Figure 5.5).

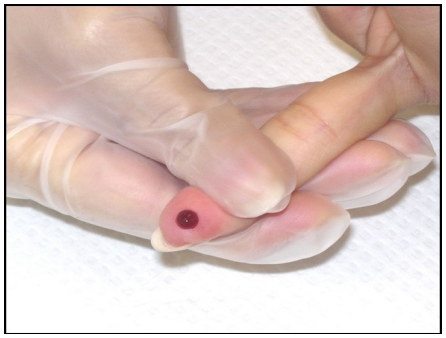


Figure 5.5 Large drop of blood

- Step 2: Position of Filter Paper Card

  - Move the card underneath the finger, with the pre-printed side of the card facing the pricked finger.

  - The card must not be pressed against the prick site on the finger. Make sure that the respondent's finger does not touch the card at any point when you are collecting the blood spots.

- Step 3: Blood Collection on the card:

  - Let the blood drop fall freely in the center of the pre-printed circle. In case the blood drop does not fall readily, you may touch the filter paper gently against a LARGE blood drop (but not the skin). In one step, a sufficient quantity of blood should be allowed to soak through and completely fill the circle.

  - You must continue to collect drops of blood until you have fully saturated three circles on the filter paper card (Figure 5.6).

  - To enhance blood flow, gently apply intermittent pressure to the area surrounding the prick site to get a third drop. Allow sufficient time for a large blood drop to form before filling the next circle on the filter paper card. Again, avoid milking or squeezing the finger.

  - If the blood flow stops or decreases before you fully saturate three circles, you will need to do another finger prick. Whenever this

Figure 5.6 Fully-filled circles without layering or caking

is necessary, you should explain to the respondent that you were unable to obtain an adequate sample and ask permission to obtain blood from another finger. Use fresh supplies and a **different finger** for the second finger prick.

- After collecting three spots on the filter paper card, place the filter paper card with the blood spots on the absorbent paper sheet away from other items. The next blood drop will be collected for anemia testing. Be careful not to drop the completed filter paper card.

Before conducting the anemia testing, first wipe away any excess blood on the skin of the finger. Next, apply gentle pressure to the puncture site to promote blood flow and then fill the microcuvette with blood to measure the Hb level.

Figure 5.7 Filling the

10. Collect the capillary blood in the microcuvette:

    - Apply the tip of the HemoCue microcuvette to the middle of the blood drop. The microcuvette chamber will fill itself automatically by capillary action. The chamber needs to be filled completely (Figure 5.7). Never "top off" the microcuvette. Instead, if the microcuvette is not completely filled, use a fresh microcuvette and fill it with the next blood drop that forms.

    - Wipe any surplus blood off both sides of the microcuvette "like butter from a knife," using the clean end of a sterile gauze pad. Ensure that no blood is sucked out of the microcuvette when wiping it – do not let the tip of the filled microcuvette touch the gauze.

    - After filling the chamber, the microcuvette needs to be visually inspected for air bubbles. Since air bubbles may influence the hemoglobin measurement, any microcuvette containing air bubbles must be discarded. In such cases, with the permission of the parent/ responsible adult, repeat the blood drop collection using a different finger (heel). Again, you must use new supplies and follow all of the steps described previously in obtaining the new sample.

Figure 5.8 Placing the microcuvette in photometer

    - Place the microcuvette in its holder and gently push the holder into the photometer (Figure 5.8).

11. If possible, continue to fill the remaining two circles on the filter paper card with blood (Figure 5.9). If not possible, stop bleeding at the site of the prick

Figure 5.9 Filling up the pre-printed circle on the filter paper card

   - After the blood drop collection, wipe any remaining blood from the prick site with a sterile gauze pad. Press the gauze pad against the prick site until the blood flow has stopped completely.

   - Take an adhesive bandage from its wrapper and apply it to the prick site.

12. Reading the results: The microcuvette should be analyzed immediately, and no later than ten minutes after being filled. The blood hemoglobin level in grams per deciliter (g/dl) is displayed 15 to 45 seconds after the drawer is closed (Figure 5.10).

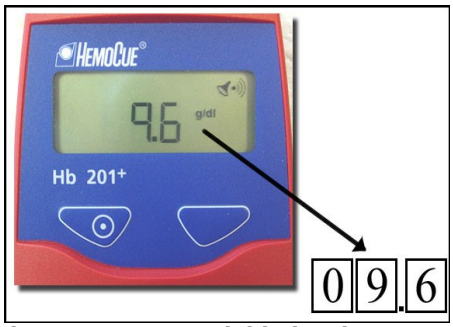


Figure 5.10 Hemoglobin level displayed on the HemoCue photometer and as it should be

13. Record the **hemoglobin level** in **Question 240/268**. If a hemoglobin measurement was not obtained because the respondent was not present, did not consent to the test, or there was a technical problem with the testing, circle the appropriate code.

14. Placing the completed filter paper card in the drying box:

   - The drying box should be placed vertically on a flat surface at the time you set up your equipment. The position of the drying box is especially important anytime it contains filter paper cards that have not completely dried. Keep the box vertical whenever it contains blood spots that are not fully dried to prevent blood that has not yet completely dried from spreading.

   - Keep the drying box closed during blood collection to prevent dust and dirt from entering the box or from contaminating DBS cards that were collected earlier and are already in the box. After the blood collection process is finished, open the drying box (keeping it vertical). Carefully pick up the completed filter paper card and place it in a horizontal position in one of the slots in the drying rack in the box. The blood spots should face towards the back of the drying box. Close the box.

   - ***Avoid touching or smearing the blood spots on other cards already in the box when you are storing a new card. Never***

put more than one completed filter paper card in a single slot in the drying rack.

- Allow blood spots to dry overnight at ambient temperature. The box should be handled carefully so that the cards do not fall out of their slots.

15. Collect biohazardous waste

    - Place all biohazardous waste (lancets, alcohol swabs, gauzes, and gloves) into a clearly labeled “biohazard” plastic bag, which has been provided for field disposal of these items. At the end of the day, follow the procedures described in Chapter 6 for the proper disposal of waste materials.

16. Record the respondent’s hemoglobin level in the anemia brochure.

17. Inform the respondent of her/his hemoglobin level and provide the anemia brochure.

18. Provide a written referral to a health facility for treatment for any respondents with severe anemia (below 9 g/dl for men and pregnant women and below 7 g/dl for women who are not pregnant or don’t know if they are pregnant).

19. Record the result for blood collection for HIV testing in **Question 241/269.**

20. Provide an informational brochure on HIV/AIDS. The brochure will include a voucher for free HIV VCT services, and a list of the nearest participating VCT centers in [COUNTRY].

Summary of the steps involved in collecting blood for HIV testing and for anemia testing for adults:

- Prepare Biomarker Data Form for individuals eligible for testing;
    - Seek voluntary consent for anemia testing and blood collection for HIV testing from the respondent (if the respondent is age 15 to 17 and unmarried seek voluntary consent from the parent/responsible adult and the respondent).
- Place the bar code labels on a filter paper card, the Biomarker Data Form, and the DBS Transmittal Sheet;
- Clean and prick the respondent's finger with an adult lancet;
- Wipe away the first drop of blood;
- Fill the pre-printed circles on the filter paper card with the second, third, and fourth drops of blood – you will fill 3 circles on the filter paper card;
- Wipe away any excess blood on the skin;
- Collect the fifth blood drop in a microcuvette;
- Collect the sixth and seventh blood drops on the filter paper, if possible;
- Stop the bleeding at the prick site;
- Test the blood sample with the HemoCue photometer;
- Record the hemoglobin level on the Biomarker Data Form
- Place the filter paper card in the drying box;
- Collect biohazardous waste.
- Inform the respondent of his/her hemoglobin level and provide an informational brochure on anemia;
- Provide a written referral for follow-up medical attention for respondents found to be severely anemic;
- Provide all respondents with an informational brochure on HIV/AIDS, vouchers for free VCT services, and a list of nearby VCT centers.

APPENDIX C: DRIED BLOOD SPOT (DBS) TRANSMITTAL SHEET

Dried Blood Spot (DBS) TRANSMITTAL SHEET (FRONT)

KEEP IN LARGE ZIPLOC BAG WITH SAMPLES UNTIL FINAL SIGNATURE OBTAINED

TECHNICIAN NUMBER ☐☐☐ CLUSTER NUMBER ☐☐☐

PERSON SENDING/ RECEIVING SAMPLES	TIME TO FILL IN FORM	TOTAL COUNT OF BLOOD SAMPLES	SIGNATURE CONFIRMING THAT EACH BLOOD SAMPLE IS PRESENT —SEE BACK OF FORM	SIGNATURE CONFIRMING THAT THE NUMBER OF BLOOD SAMPLES MATCHES COL. 3	DATE	NOTES (NOTE ANY DISCREPANCY IN NUMBERS OF SAMPLES)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
TECHNICIAN	WHEN CLUSTER IS COMPLETED	☐☐				
FIELD TEAM SUPERVISOR	AFTER TECHNICIAN HAS DONE HIS/HER COUNT	☐☐				
SAMPLE PICK UP PERSON	WHEN SAMPLES ARE PICKED UP IN FIELD	☐☐				
RECEIVER AT [IMPLEMENTING AGENCY]	UPON ARRIVAL AT [IMPLEMENTING AGENCY]	☐☐				
RECEIVER AT LABORATORY	UPON ARRIVAL AT LABORATORY	☐☐				

TECHNICIAN: Upon completion of a cluster, verify that the bar code number on each blood sample (filter paper card) collected and stored in the large zip-loc bag labeled with the cluster number corresponds to a bar code number pasted to the back of this transmittal sheet (and vice-versa). Note any discrepancies in Column (7). Count and record the total number of blood samples in Column (3). Sign your name in Column (4) and the date in Column (6). Fold and store this transmittal sheet in the large zip-loc bag.

FIELD TEAM SUPERVISOR: After the technician has verified the blood samples, you will conduct a second verification. Count the number of blood samples, record the total in Column (3). Verify that the bar code number on each blood sample (filter paper card) collected and stored in the large zip-loc bag labeled with the cluster number corresponds to a bar code number pasted to the back of this transmittal sheet (and vice-versa) and sign Column (4). Verify that your count of blood samples is the same as the number reported by the technician and sign Column (5). Note any discrepancies in Column (7). Record the date in Column (6). Fold and store this transmittal sheet in the large zip-loc bag.

SAMPLE PICK UP PERSON: Before leaving a cluster to return to [Implementing Organization], you will verify the number of blood samples collected in the completed cluster. For the completed cluster, count and record in Column (3) the total number of blood samples stored in the large zip-loc bag labeled with that cluster number. Note any discrepancies in Column (7). Sign your name in Column (5) and the date in Column (6). Fold and store this transmittal sheet in the large zip-loc bag.

AT THE IMPLEMENTING AGENCY OFFICE: For each large zip-loc bag arriving from the field, you will verify the number of blood samples received. Count and record in Column (3) the total number of blood samples stored in the large zip-loc bag labeled with the cluster number. Note any discrepancies in Column (7). Sign your name in Column (5) and the date in Column (6). Fold and store this transmittal sheet in the large zip-loc bag.

RECIEVER AT THE LABORATORY: Upon receiving blood samples from [IMPLEMENTING AGENCY], verify that the bar code number on each blood sample (filter paper card) collected and stored in the large zip-loc bag labeled with the cluster number corresponds to a bar code number pasted to the back of this transmittal sheet (and vice-versa). Note any discrepancies in Column (7). Count and record the total number of blood samples in Column (3). Sign your name in Column (4) and the date in Column (6). Photocopy both sides of this form and return the original to [Implementing Agency].

Note: This form will be destroyed under the direction of the Lab Director after all blood sample have been completely processed and a final HIV test result has been determined for each usable sample.

DBS TRANSMITTAL SHEET (BACK)

NO.	SAMPLE BAR CODE	TECH-NICIAN	LAB
1			
2			
3			
4			
5			
6			
7			

NO.	SAMPLE BAR CODE	TECH-NICIAN	LAB
16			
17			
18			
19			
20			
21			
22			

Fold here

8			
9			
10			
11			
12			
13			
14			
15			

NO.	SAMPLE BAR CODE	TECH-NICIAN	LAB
23			
24			
25			
26			
27			
28			
29			
30			

APPENDIX D: VCT VOUCHER

VCT

Voluntary Counseling and HIV Testing (VCT)

Introduction

HIV is the virus that causes AIDS and may be transmitted through sex or contact with blood or from a mother to her baby. Confirming whether one is free or is already infected with this virus is a crucial step towards a confident future.

Why go for counseling and HIV testing?

There are many reasons why you should go for voluntary counseling and HIV testing:

- You have questions or worries about AIDS. Perhaps someone you know has the disease. Perhaps you simply want to know how to protect yourself.
- You or your partner may have been exposed to HIV virus without knowing. Many people who have HIV look healthy and do not know they have the virus. Often when a person is infected, their partner or spouse may still be uninfected.
- You and your partner may be planning to have a baby. Treatment is available to prevent a mother from passing the virus to her baby.
- You or your partner may have had symptoms or a disease that made you worried about AIDS. A VCT counselor will answer your questions about HIV and AIDS so you can protect yourself and your family. You may go alone or go with your partner and learn your results together.
- Testing is voluntary — it is your choice whether to be tested.
- VCT is anonymous — no one will take your name and your privacy will be protected.
- Testing is rapid — you will generally receive your results within the same hour.
- Two different, high quality tests are used to give you full confidence in your results.

Where to go for HIV counseling and testing?

VCT services are now available in many areas of [COUNTRY].

The list on the back will tell you where you can go for VCT. Please present this brochure to the VCT centre staff to guarantee that VCT services are free for you and your partner.

END OF BIOMARKER FIELD MANUAL.